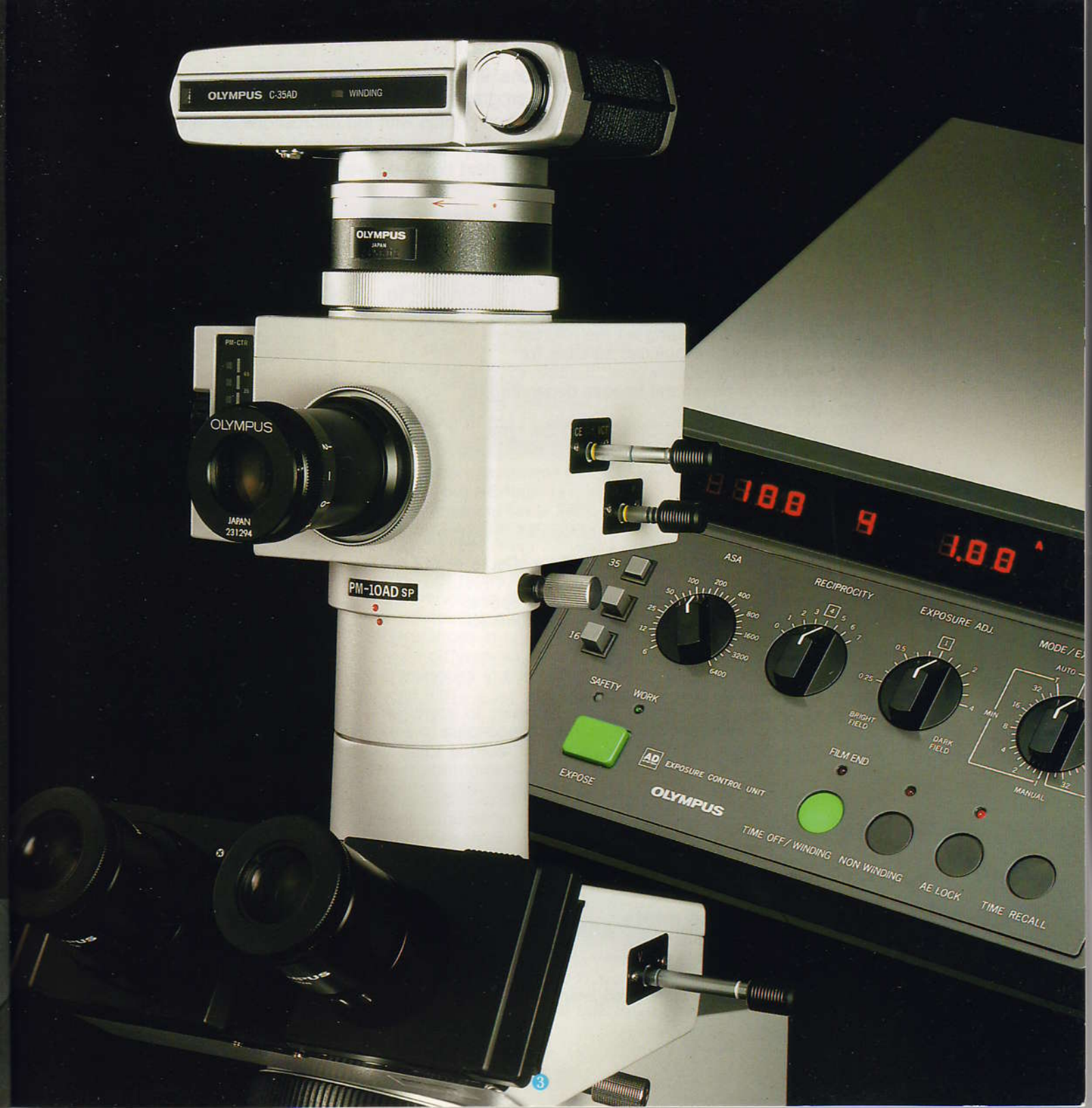
How to Improve Photography Through the Microscope

Introduction

This booklet is intended for people who may have used a microscope before, but have never taken any photos with it, as well as for those who have, but are far from satisfied with the results of their labor. By presenting the information both as illustrations and as text, the brochure will teach you how to get good photos through the microscope without any foul ups. As a result of the tremendous progress made in photomicrographic equipment in recent years, anybody can now quite easily take pictures when it comes to photomicrography, just as with a normal camera. But in order to ensure that you get satisfactory photos, you must properly adjust the equipment and follow the established procedures. To familiarize yourself with the actual techniques one by one, please consult this brochure regularly. We would be very happy if it can help you to reach a level of proficiency in photomicrography where you can confidently take good photos with the microscope, on a routine basis.

The explanations in this booklet are based on the Olympus micro-microscope model BHS and on the models PM-10AD and PM-10ADS photomicrographic equipment.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WINDING
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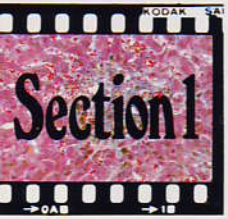
231294
PM-10AD SP
ASA
RECIPROCITY
EXPOSURE ADJ.
MODE / EX
AUTO
MANUAL
SAFETY
WORK
EXPOSE
AD EXPOSURE CONTROL UNIT
OLYMPUS
FILM END
TIME OFF / WINDING
NON WINDING
AE LOCK
TIME RECALL
BRIGHT FIELD
DARK FIELD

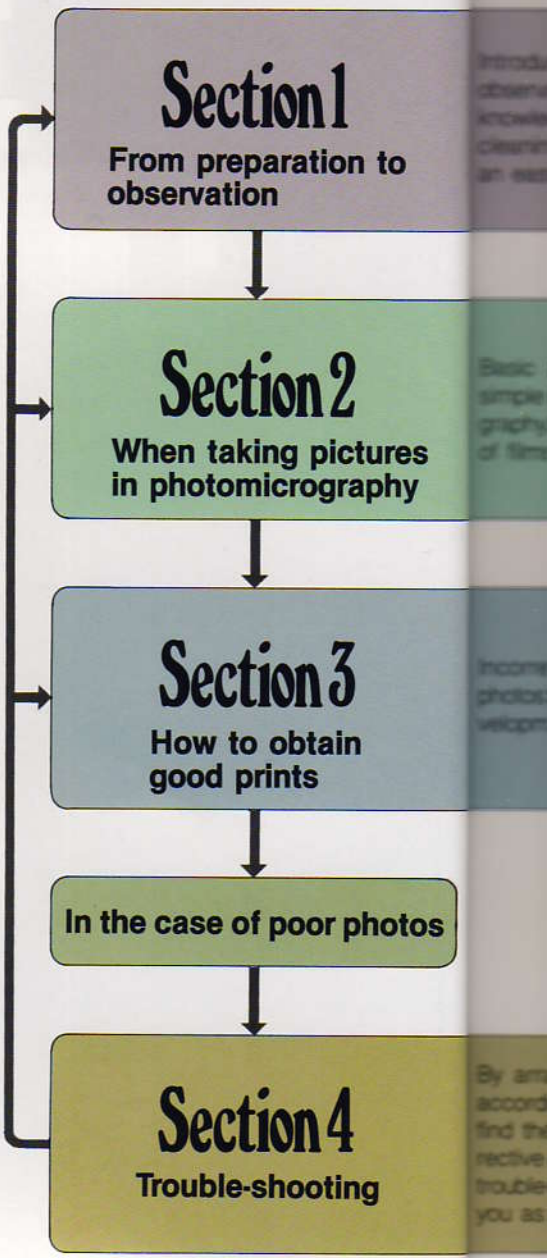
How to get the most out of this booklet

This booklet is divided into four main sections. Items marked with a ● list the minimum information that newcomers to the art of photomicrography need to know, while items left unmarked are addressed to users who have already taken photos with the microscope but are not satisfied with the results. By referring to sections 1 to 3 for picture taking and section 4 for checking, you will find this brochure a reliable help the next time you want to take a photomicrograph.



From preparation to observation

How to obtain good prints



Introduction of equipment suitable for observation and photomicrography, basic knowledge, observation procedures, cleaning methods, etc. are explained in an easy-to-understand manner.

Basic knowledge and explanation of simple procedures needed for photography, differences in films and purchase of films, etc.

Incorrect processing can ruin the best photos! This section deals with film development, how to order color prints, etc.

By arranging finished photomicrographs according to their characteristics you can find the causes for failure and take corrective action. In many instances, the trouble-shooting section can also serve you as a check-list.

When taking pictures in photomicrography

Basic information in photomicrography

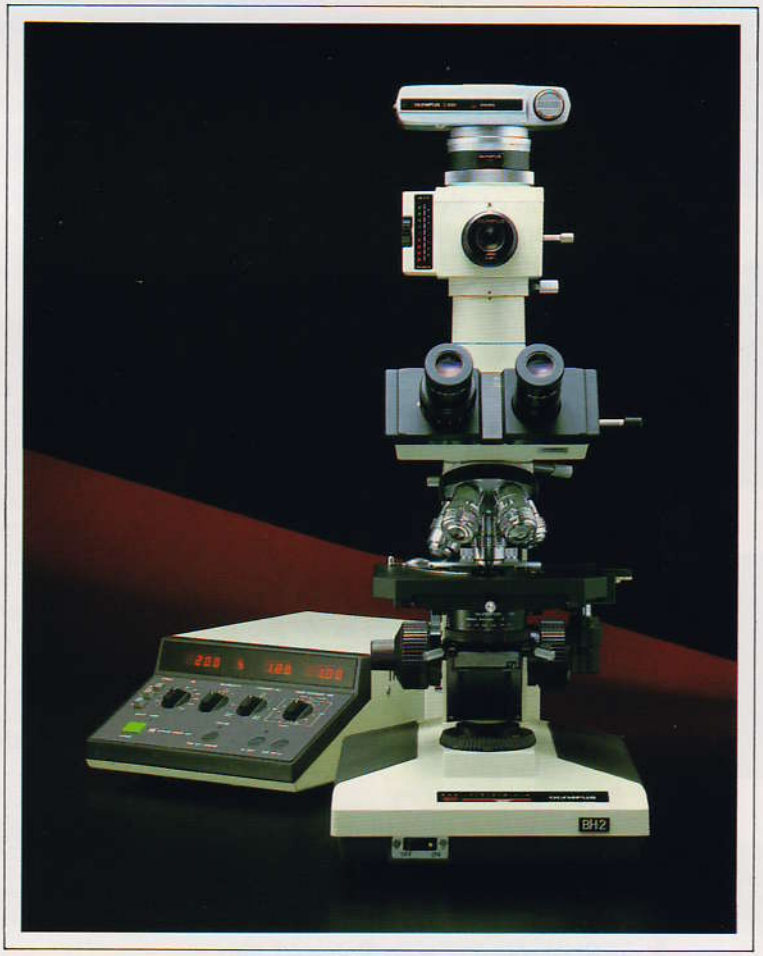
Photomicrography techniques

Color photomicrography

Black-and-white photography

Trouble-shooting

Problems in finished photos and their correction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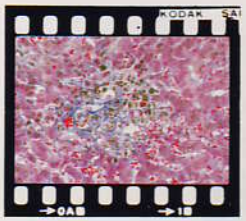
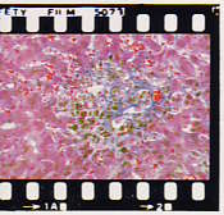


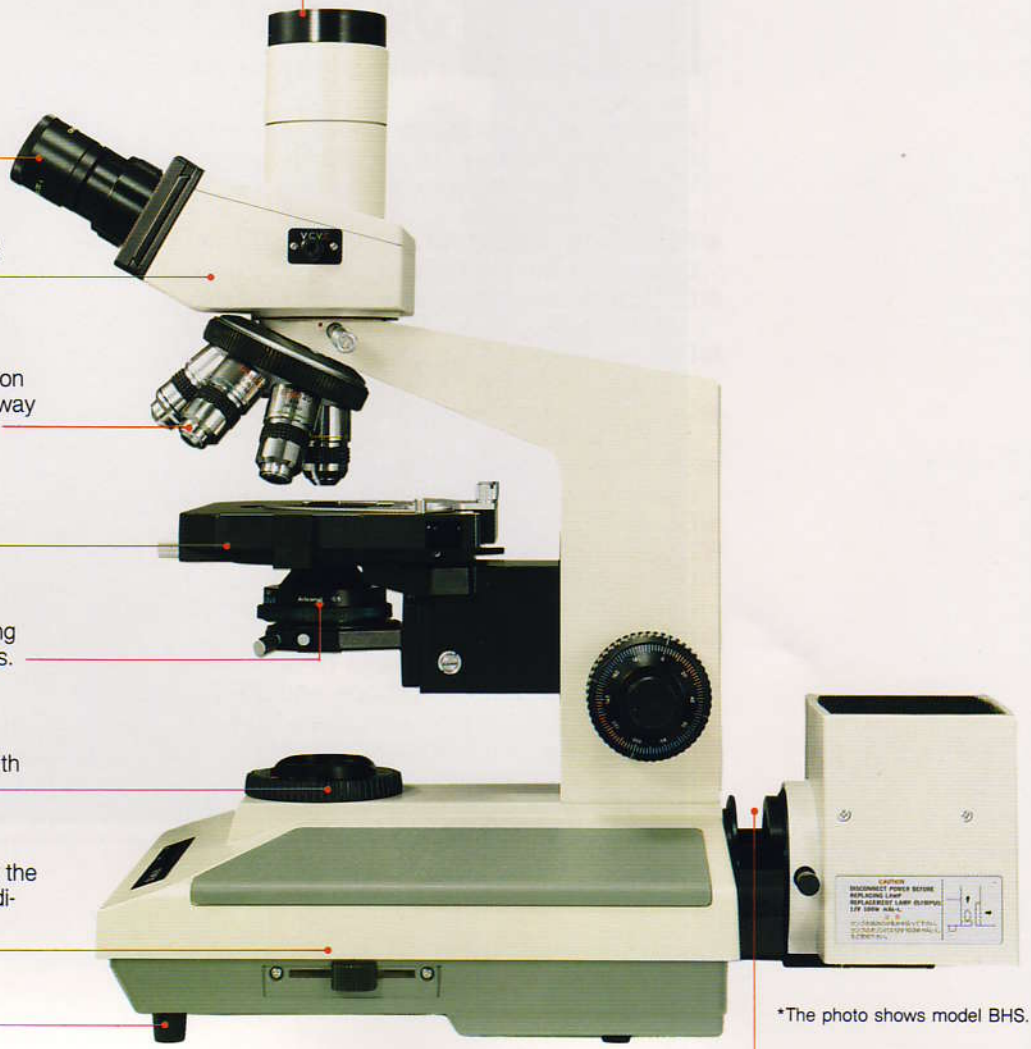
Section 1

From preparation to observation

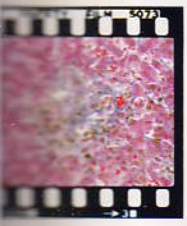


Microscopes suitable for photomicrography

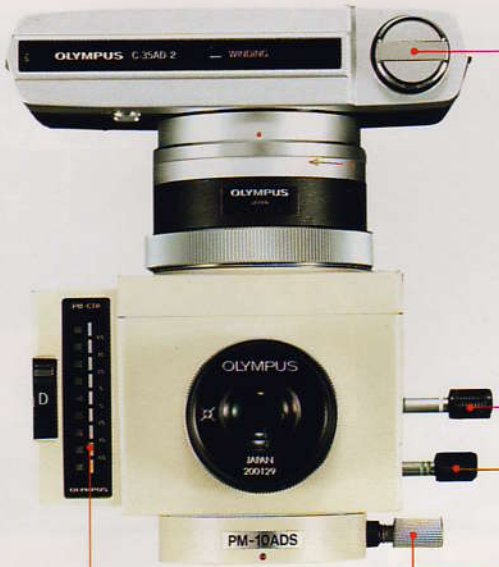
1. The phototube accepts the photomicrographic camera attachment.
2. The image can be focused through the binocular tube.
3. The desired light intensity matching the specimen conditions can be set by the optical path selector.
4. Objectives should have high resolution and good flatness (flat image all the way to the periphery of the visual field).
5. The stage is rotatable.
6. The condenser is equipped with an aperture iris diaphragm and centering device for aligning to the optical axis.
7. The microscope is also equipped with a field iris diaphragm.
8. Sufficient light intensity is assured. Light intensity is adjustable to match the observation and photographing conditions. Uniform illumination from low to high magnification.
9. The microscope stand is resistant against external vibrations.
10. Provision for insertion of filters.



*The photo shows model BHS.



Equipment suitable for photomicrography



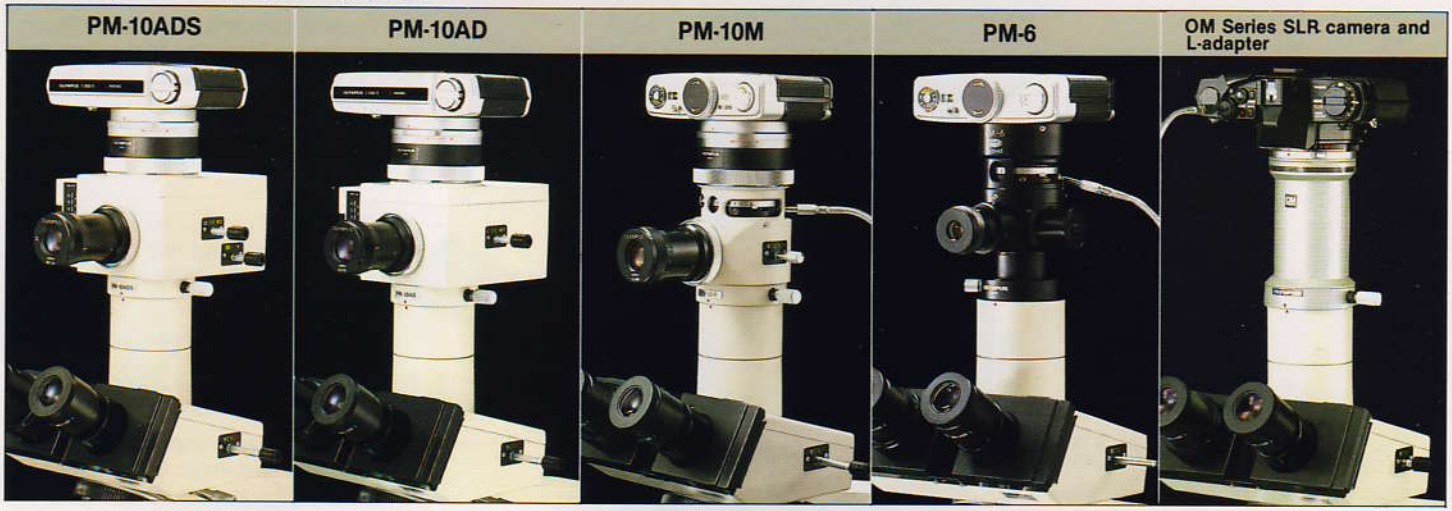
1. The camera attachment accepts 35mm or large-format films. The 35mm camera back is equipped with automatic film advance.
2. The optical path can be changed to match photographic conditions.
3. The measuring area can be changed to match the specimen. (Integrated metering 30%—spot metering 1%)
4. The camera attachment is firmly clamped on the microscope.
5. Device for measuring color temperature.

6. Wide range for setting ISO/ASA sensitivity.
7. Compensation for reciprocity law failure is carried out automatically for long exposure.
8. Exposure adjustment, matching specimen conditions, is possible.
9. Manual exposure is possible.
10. Special applications, such as 16mm cine and 35mm time lapse photography, can be performed.
(A special control unit is required in these cases.)
11. An exposure lock mechanism (AE lock) is built-in.

*The photo shows model PM-10ADS.

Various types of photomicrographic equipment and performance

Types of photomicrographic equipment



	PM-10ADS/ PM-10AD	PM-10M	PM-6	OM Series SLR camera and L-adapter
Ease of focusing	○	○	○	The focusing screen must be replaced for photomicrography*1
Photographic quality	○	○	○	Shutter blur may result when 40X and 100X objectives are used*2
Ease of operation	○	●Manual winding of 35mm film ●Use of cable release		●Manual winding of film ●Use of cable release ●Use of Varimagni Finder for focusing
Exposure measurements	○	Use of exposure meter (EMM-7)		●Use of built-in exposure meter
Use of large format film (4″×5″ sheet, Polaroid®)	○	○	×	×

*1 Concerning the focusing screen for Olympus OM System cameras:
The screen can be changed to suit different uses (OM-1, OM-2, OM-3, OM-4). For photomicrography use screen No. 1-12.

*2 Shutter blur may occur if magnification is increased. When using 40X and 100X objectives, adjust the light intensity so that the shutter speed is 1～2 sec. In this case, some types of color film may require color compensation.

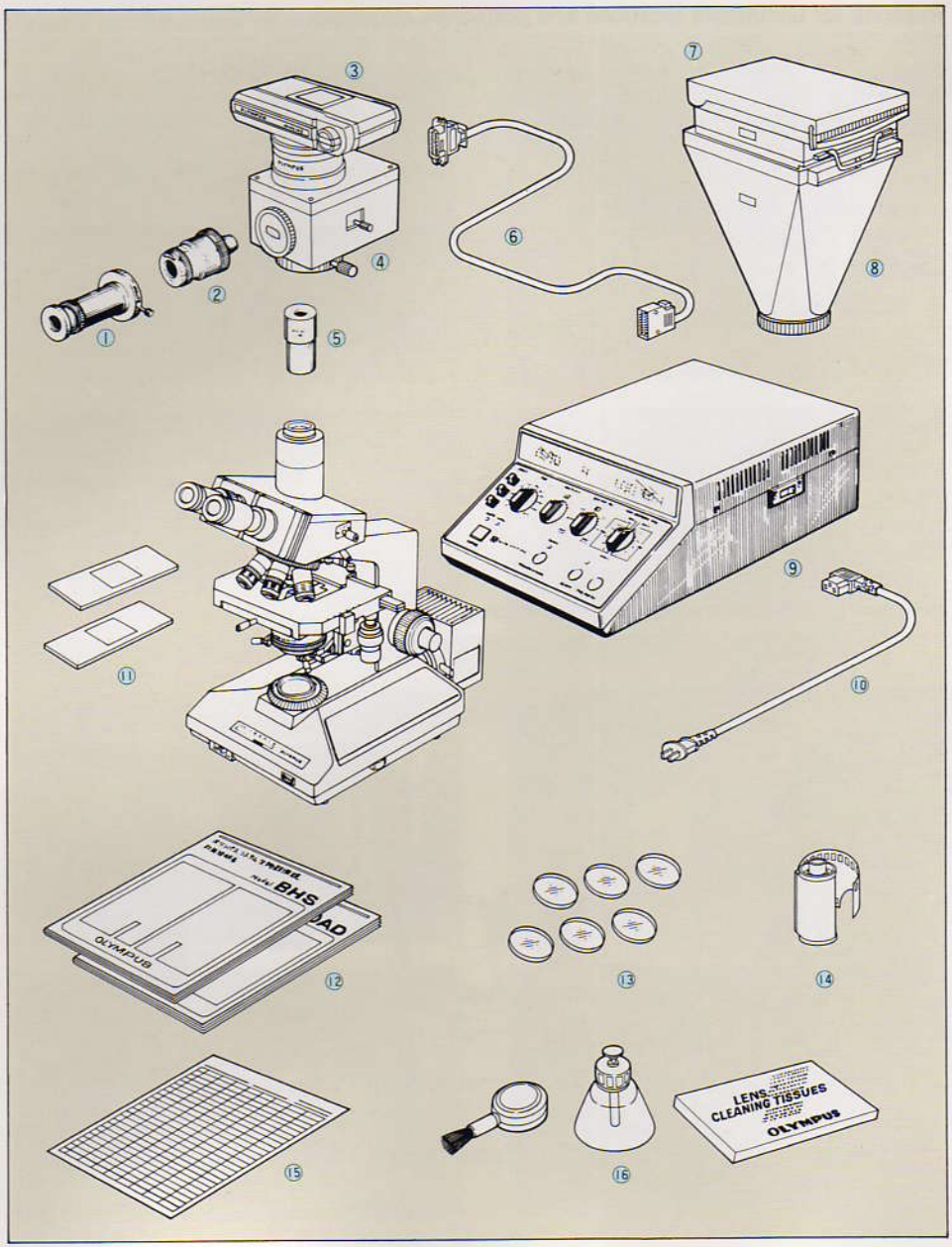
(For color compensation refer to page 53)

Equipment needed for photomicrography

First check the chart to see if the equipment required for photomicrography has been completely assembled. (The types listed below are the most basic attachments needed for taking photos of stained specimens in transmitted light.

Equipments required in photographing

1. Focusing magnifier (FT)
2. Focusing Telescope
3. 35mm camera back
4. Automatic exposure body
5. Photo eyepiece
6. Connecting cord
7. Large-format camera back
8. Adapter for large-format camera
9. Automatic exposure control unit
10. Power cord
11. Specimen
12. Instruction manuals
13. Filters
14. Film
15. Form for recording data
16. Cleaning utensils: blower, cleaning liquid, lens cleaning tissue

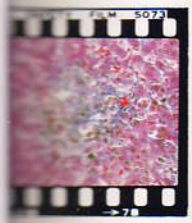


Suitable locations for setting up the microscope

Reasons for unsuitable locations and corrective measures

Unsuitable room	Consequences	Treatment
●Located too close to mechanical appliances or machinery that can cause external vibrations ●Places in which the vibration of persons walking past can be transmitted	Blurred image as a result of vibrations	●Remove the microscope from the source of vibrations ●Use a sturdy table as support ●Use a vibration-proof table
●Use of the microscope near a window	Bright light from the window prevents correct focusing	●Set up the microscope near a wall ●Position the microscope in such a way that the overhead light falls in slightly in front of the microscope.
●Place where room light enters the eyepiece	Room light or flares are reproduced on the photo	●Cover the eyepieces with caps ●Shut out stray light getting into the eyepiece or the focusing telescope by changing the optical path selector
●A dusty and dirty room ●Place near a window where dust can enter from the outside	Black spots are reproduced on the specimen image	●Set the microscope up in another room ●Cover the whole microscope with a dust-proof covering

Example of a suitable room for photomicrography



Objectives and photo eyepieces suitable for photomicrography

Objectives

For photomicrography high-resolution objectives with flatness all the way to the periphery of the visual field are required. Of the LB (long-barrel) objectives, series S Plan Apochromat, S Plan Achromat, and D Plan Achromat, and of the short-barrel objectives, Plan Apochromat and Plan Achromat types are recommended.

Photo eyepieces

The photo eyepiece is optically compensated to permit the objective to deliver its full performance on the film plane. It must be correctly matched with the objectives.

Correct combinations of objective and eyepiece

(1) Combination with NFK type

(2) Combination with FK/P types

S Plan Apochromat series

Short-barrel Plan Apochromat series

S Plan Achromat series

Short-barrel Plan Achromat series

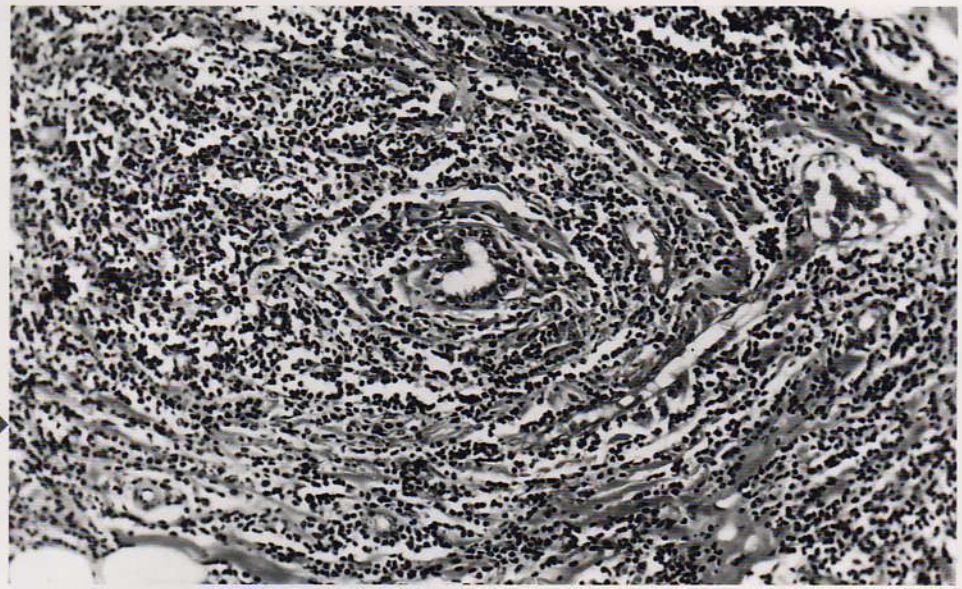
D Plan Achromat and D Achromat series

Short-barrel Achromat series

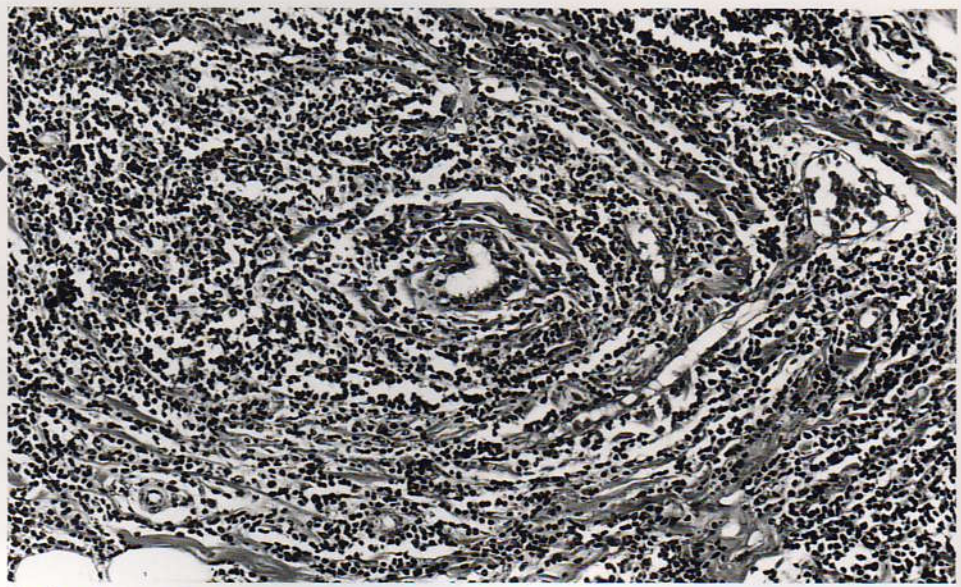
Differences in the peripheral images depending on objective design

According to the type of objective, the periphery of both the observed image and the photographed image may appear out of focus. This effect is caused by the performance characteristics of Achromat type objectives. Using Plan Achromat objectives, however, will result in a sharp and flat image extending all the way to the periphery of the field of view.

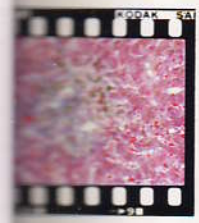
D Achromat 10X, NFK 3.3XLD



D Plan Achromat 10X, NFK 3.3XLD



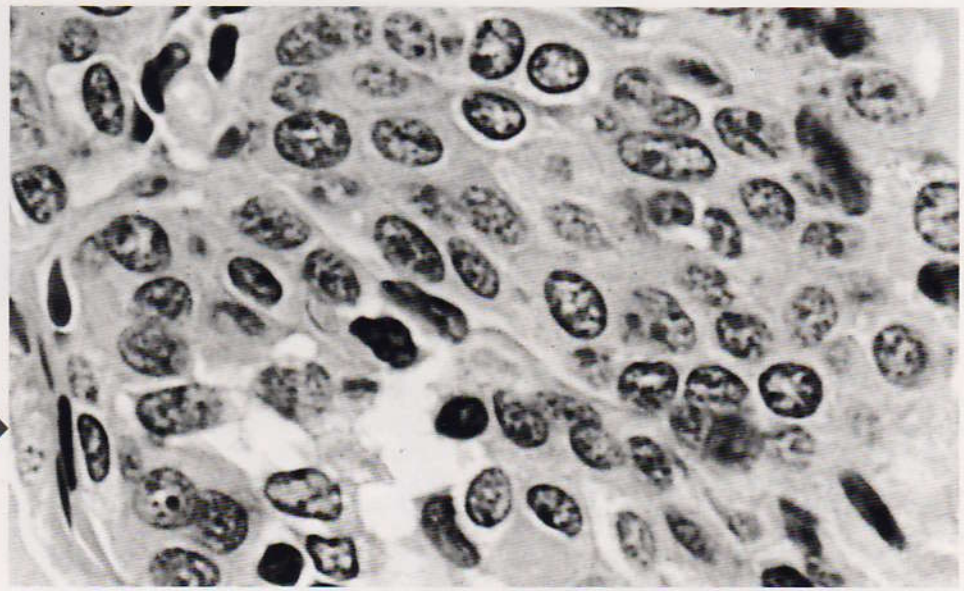
The field flatness of a D Plan Achromat objective extending all the way to the periphery is superior to the one provided by a D Achromat objective.



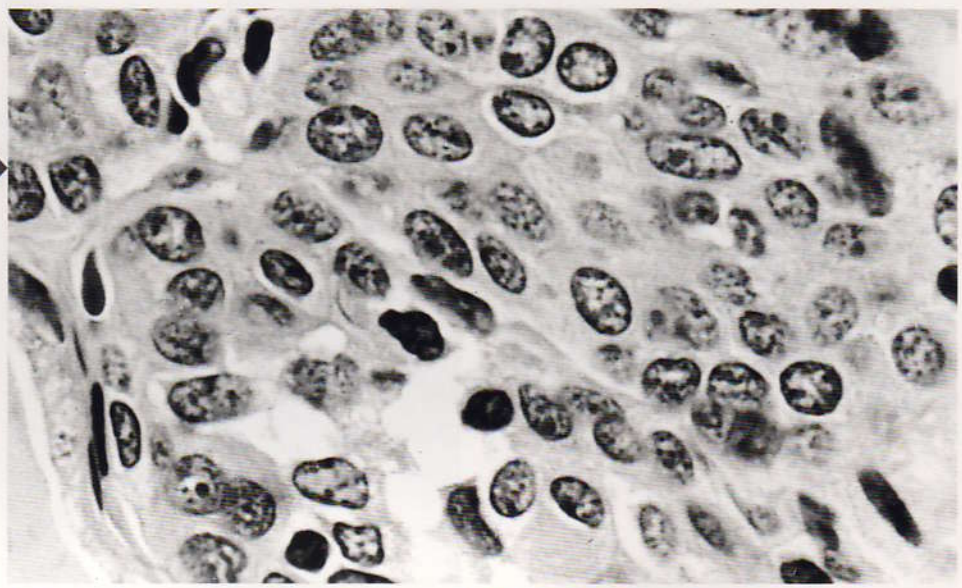
Difference in resolution depending on the type of objective

Resolution in the center of the field of both the observed and the photographed images differs according to the type of objective. The top class objective series, S Plan Apochromats, as well the S Plan Achromat series provide superior resolution.

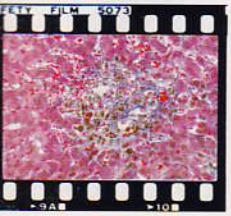
D Plan Achromat 40X, NFK 3.3XLD



S Plan Apochromat 40X, NFK 3.3XLD



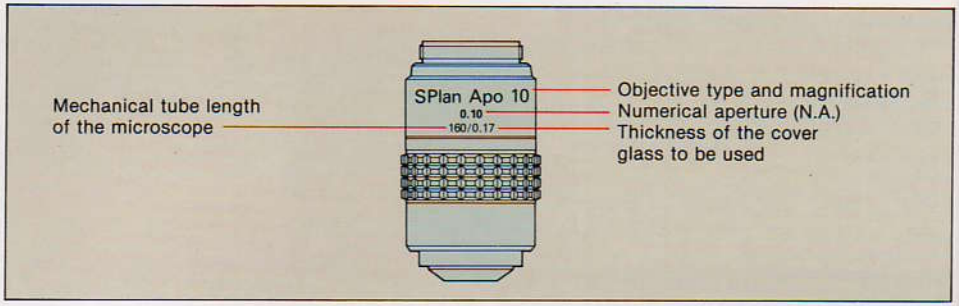
Gives sharp resolution of the whole image, allowing observation of minute details.



Some hints about the objective

It is important to choose an objective suitable for your specific purpose

For all objectives, proper use depends on the specific purpose, some types requiring some adjustments. In order to fully utilize the objective, you should know the meaning of the various numbers and letters engraved on the objective barrel.



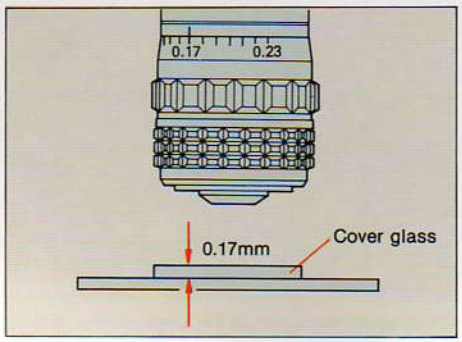
1. Objective with correction collar

In objectives with a numerical aperture (N.A.) above 0.6—excluding oil-immersion types—the thickness of the cover glass strongly affects the image quality. Cover glass thickness is theoretically designated at 0.17mm, although in actual practice this may vary by ±0.3mm. By optically compensating for this thickness deviation, the correction collar assures the best image.

Method of adjustment

(1) Set the scale to the 0.17mm position, then focus.
(2) Rotate the correction collar 2 or 3 graduation marks (0.02-0.03mm) in the direction of 2, and refocus. If the image is sharper than under (1), rotate the collar another 2 or 3 graduation marks in the same direction and focus again.
(3) If you are not satisfied with the image quality, try rotating the collar in the opposite direction for 1-2 graduation marks, refocusing and comparing the image.
(4) Gradually reduce the amount of rotation of the correction collar, and try to find the optimum condition by repeating steps (2)—(3).

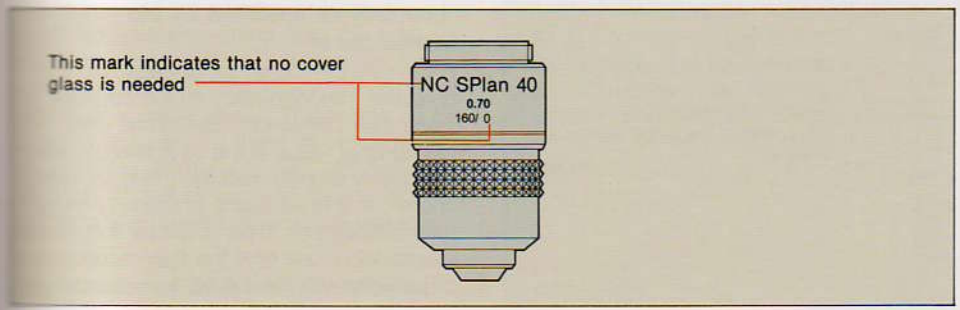
- The above adjustment procedures must be repeated every time the specimen is changed.



2. Objective with iris diaphragm

Some objectives above 40X are equipped with an iris diaphragm. This diaphragm prevents direct light from entering the objective in darkfield or transmitted light fluorescence observation. By watching both contrast and resolution of the image, the aperture can be adjusted to the optimum position.

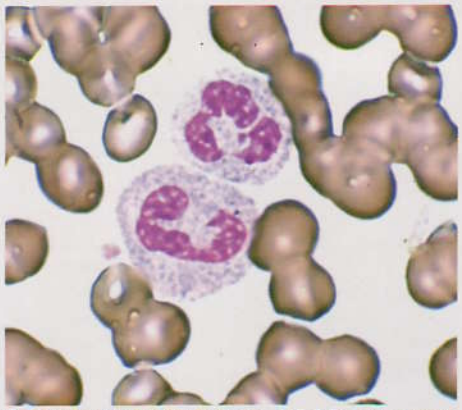
D Plan 50X, S Plan Apo 100X, NC S Plan Apo 100X



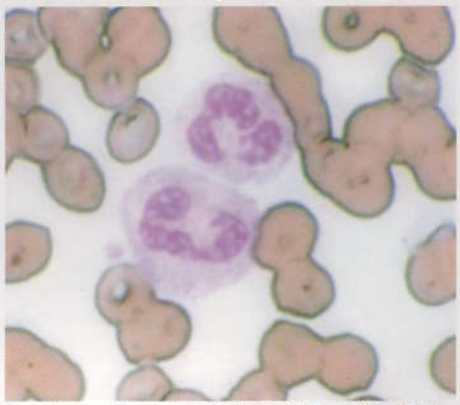
1. No-cover (NC) objective

For specimens without cover glass such as smears, objectives bearing the mark NC or the cover glass affects the image clarity, a fact which is particularly obvious in the case of objectives with a large numerical aperture. As a result, when observing specimens not protected by a cover glass at high magnification, a special NC objective is used. When viewing uncovered specimens through normal objectives, only poor images with many flares and insufficient resolution can be obtained.

The Olympus LB series no-cover objectives: NC S Plan 40X, NC D Plan FL 60X, NC S Plan Apo 100X, and NC S Plan 100X dry



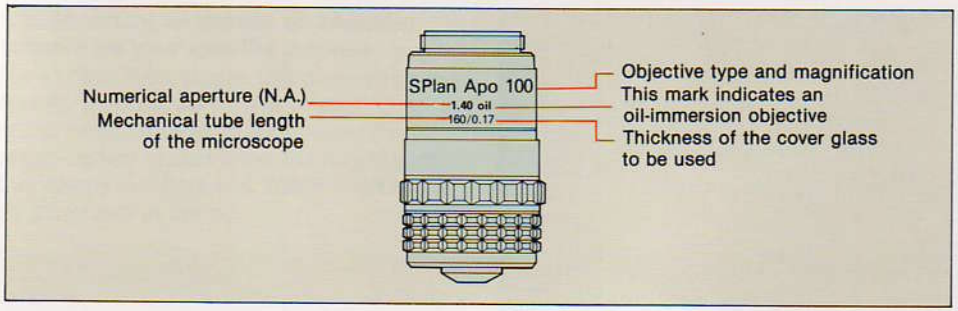
Specimen without cover glass—NC S Plan Apo 100X



Specimen without cover glass—S Plan Apo 100X

4. No-cover 100X dry objective

High-magnification 100X objectives are usually of the oil immersion type, but for the no-cover 100X objective a dry type is available. Using this objective, together with other non-immersion objectives, e.g. no-cover 40X and 60X, obviates the need to put immersion oil on the specimen slide. The 100X dry-type lens can also be used for photomicrography, but for achieving optimum picture quality, the use of an oil-immersion objective is recommended.



***Use only oil specified by the manufacturer**

If you use old Cargill oil or cedar oil, the objective cannot display its full potential, since their diffraction coefficients differ from the nominal value. If the oil is tinted, it affects the quality of color rendition in color photos. Therefore, it is advisable to use only manufacturer-specified oil. In particular for fluorescence examination, use only the fluorescence-free oil provided with the fluorescence microscopes.

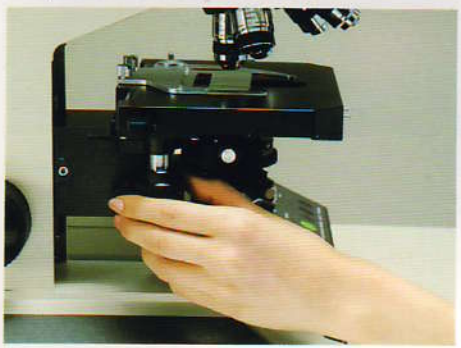
5. Oil-immersion objective

Use of the oil-immersion objective

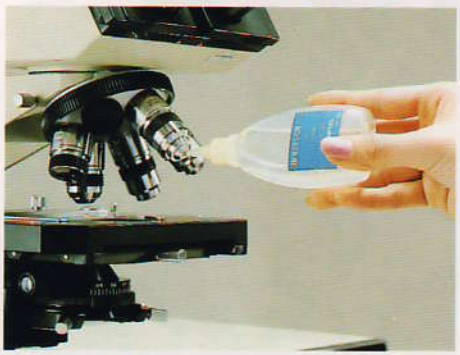
Oil-immersion objectives have a numerical aperture above 1.0 and use manufacturer-specified oil between the front lens of the objective and the specimen. (Oil-immersion objectives carry the mark "oil".) In order to make full use of the resolving power of the objective, it is preferable to use oil also between the condenser front lens and the specimen slide.

How to apply the oil

(1) Focus on the specimen, with the 10X, or 40X objective and bring the desired specimen detail in the field of view.



(2) Rotate the revolving nosepiece so that the oil-immersion objective is pointing towards you, and apply oil to the front lens of the objective.



(3) Apply oil to the specimen surface and rotate the nosepiece until the oil-immersion objective, its tip likewise covered with oil,

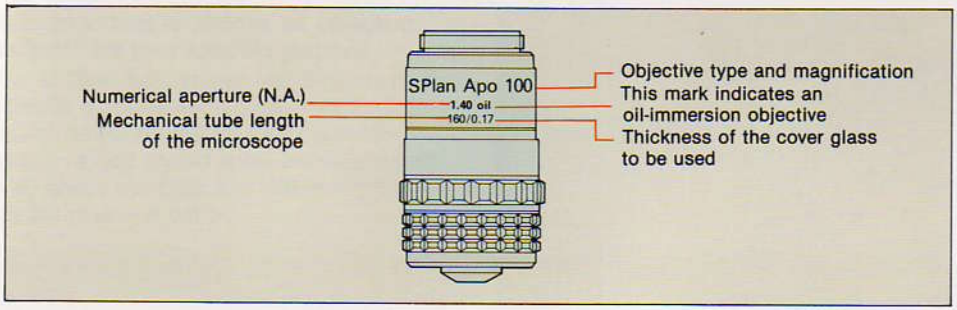
clicks into the light path. Make sure that the objective front lens is fully immersed into the oil on the specimen slide.

(4) If the image is not in view, slowly rotate the fine focusing knob till it comes into focus.

■ Make sure that the objective front lens does not get too close to the specimen, since the image will deteriorate if air bubbles get into the oil. If a haze seems to cover parts of the image, even though it is in focus, swing the nosepiece 1 or 2 times from the click stop in order to remove the air bubbles.

If the image still does not improve, remove the eyepiece as illustrated above, check for air bubbles by viewing the back lens of the objective, wipe off the oil and reapply oil.

(For the method on how to clean an oil-immersion objective, refer to page 32)



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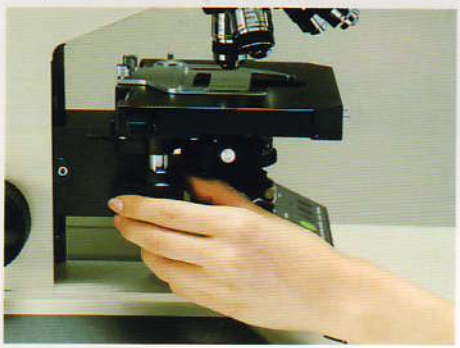

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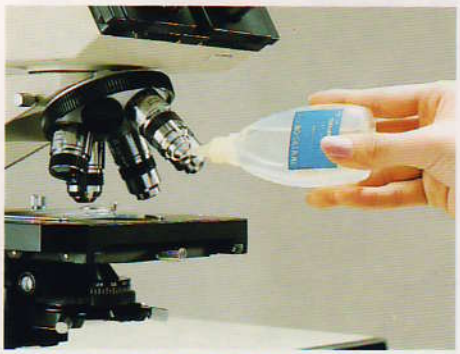

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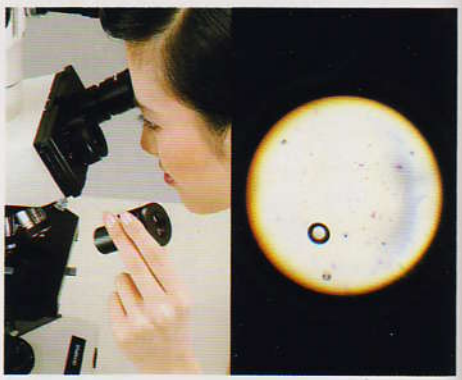
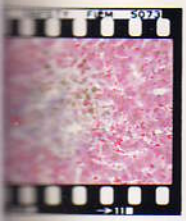


If the image still does not improve, remove the eyepiece as illustrated above, check for air bubbles by viewing the back lens of the objective, wipe off the oil and reapply oil.

(For the method on how to clean an oil-immersion objective, refer to page 32)



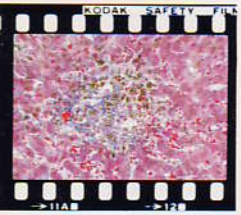
Selection of a condenser

The purpose of the condenser is to efficiently focus the light, emanating from the light source, on the specimen, to create lighting conditions matching the objective, and thus to provide a better image. Depending on the intended use, several types of condensers are available. Particularly with ultra-low magnification objectives such as 1X, 2X, and 4X, problems like uneven illumination and insufficient amount of light at the periphery are likely to occur. Therefore make sure to use these objectives in combination with the ultra-low magnification condenser.

*In order to obtain better photos with the S Plan FL 2X objective, use of the ultra-low magnification condenser BH2-ULC is recommended.

Combination of BH2 series condensers and LB objectives

Condenser		Objective used	
BH2-AAC Achromatic Aplanatic condenser		S Plan Apo, S Plan 10X ~ 100X	
BH2-SC Achromatic Swing-out condenser		S Plan FL 2X,* S Plan 4X ~ 100X	
BH2-CD Achromatic condenser		D Plan, D 4X ~ 100X	
BH2-ULC Ultra-low condenser		S Plan FL 1X, S Plan FL 2X, different design 4X objectives	



Observation procedures

Now we are finally getting to observation, but first make sure that no dust or dirt is on the objective, eyepiece, and specimen. Make it a habit to check for dirt before you use the microscope, since dirt prevents focusing and results in poor image quality.
(For dust and dirt detection, refer to page 28, for cleaning methods refer to pages 29-33)

1 Placement of filters

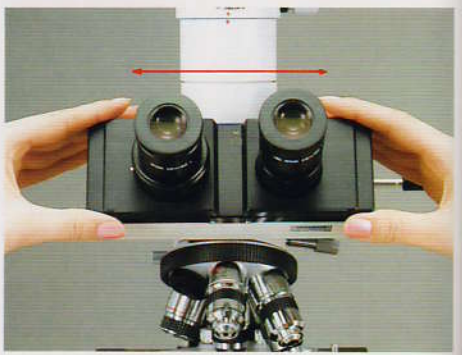
Turn on the main switch and adjust the voltage to position Photo (ca. 9V).

(1) Place the light balancing filter (LBD-2N) on the light exit window.

(2) Place an ND filter in the slot close to the lamp housing. Depending on the objective magnification and the density of the specimen, use this filter so that it provides enough brightness for easy examination.

2 Adjustment of interpupillary distance

(3) Place the specimen on the stage and focus with a 10X objective.
(For the method of focusing refer to pages 26-27)



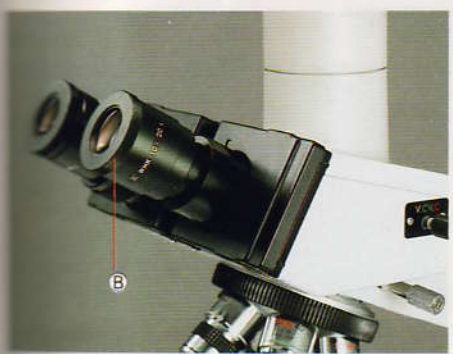
(4) Adjust the interpupillary distance until both left and right view fields merge into one.

3 Diopter adjustment

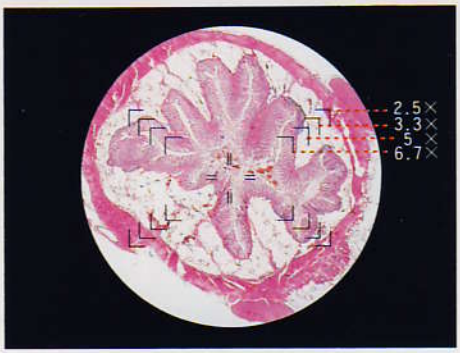
Adjust the diopter to suit the observer's eyesight. The method differs according to the eyepiece used.

■ Unless the diopter is adjusted, parfocality will not be maintained when the objective is changed.

(5) When using WHK 10X eyepieces, the focus is adjusted with the focusing knobs while observing through the right eyepiece. The diopter adjustment ring Ⓐ on the left side is then adjusted for maximum image clarity for the left eye.



(6) When using a finder eyepiece for photomicrography, adjustment is made by rotating section Ⓑ of the eyepiece at the right eye.

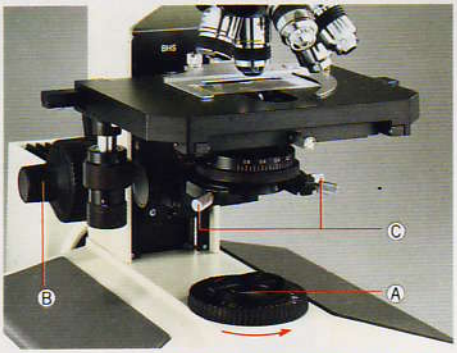


(7) At that time, adjust the image so that the cross lines in the center of the frame mask are clearly distinguished as two separate lines. Then adjust the focus by rotating the fine-focusing knob so that the cross lines and the specimen image are in focus simultaneously. After completing right-eye adjustment, also adjust the diopter for the left eye by rotating section Ⓐ as in (5).

■ Since the type of the finder eyepiece differs according to the size of the film used, choose the type suitable for your particular purpose.

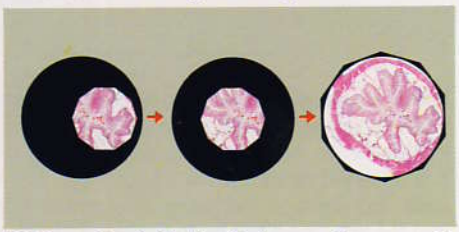
35	WHK10X	35mm film
4×5	WHK10X	4×5″ sheet film
P.	WHK10X	4 1/4×3 1/4″ Polaroid®
MH	WHK10X	16mm cine

4 Condenser centering



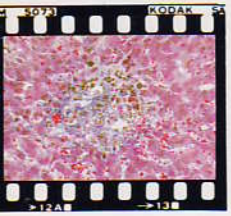
(8) After focusing on the specimen with the 10X objective, rotate the field stop Ⓐ in the direction of the arrow and reduce the field iris diaphragm diameter to a minimum. Then slowly move the condenser from top to bottom by using the condenser height adjustment knob and stop at a position where the field iris diaphragm image is sharply defined.

■ Move the field iris diaphragm image to the center of the visual field with the condenser centering knobs Ⓒ.



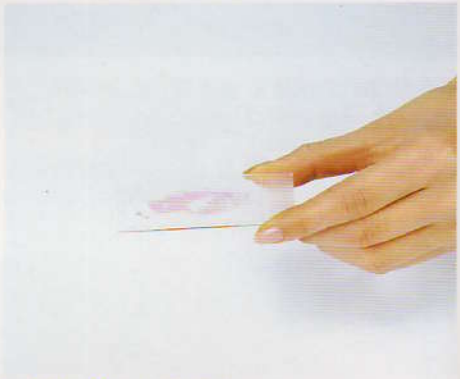
(9) Open the field iris diaphragm image until it almost touches the periphery of the visual field, and make some final centering adjustments. For normal observation conditions, make the diaphragm slightly larger than the visual field.

■ If the field iris diaphragm cannot be sharply focused, check the thickness of the specimen slide. Use slides with a thickness between 0.9 and 1.2mm.

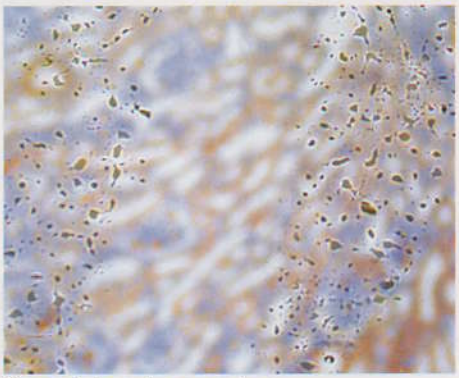


Handling of specimens

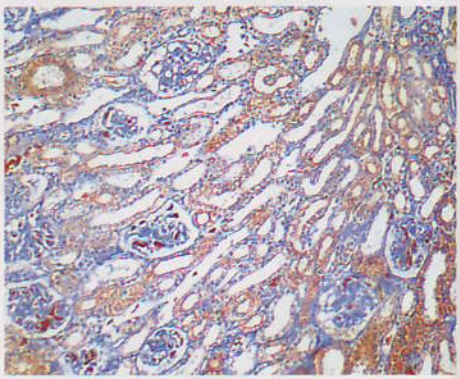
Make it a habit to clean the specimen regularly both before and after observation. Just as with lenses, it is most important to work with a clean specimen. Make sure that no dust particles stick to the specimen when you store it and do not touch the glass surfaces when you handle it.



Hold the glass slide at the corner only, or hold the glass slide between your fingers.



Fingerprints on the cover glass.



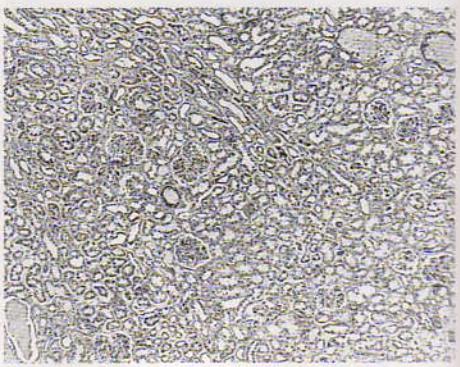
The image is blurred along the traces of your fingerprints.

Notes on specimen placement on the stage

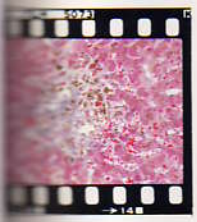
When you place a specimen on the stage, the specimen holder is opened wide, and if it is released rapidly, it will hit the edge of the slide and damage the glass. After confirming that the specimen has been firmly put in place, the spring-loaded specimen holder must be retracted slowly so that it gently touches the edge of the slide.

In case the slide is damaged, carefully remove the tiny glass fragments. If fragments are left on the stage, they may cause injuries, or the specimen slide may be placed in a tilted position on the stage, causing one side of the visual field to be out of focus.

Put the specimen firmly in place and slowly retract the specimen holder.



One side of the visual field is blurred because of a tilted slide.

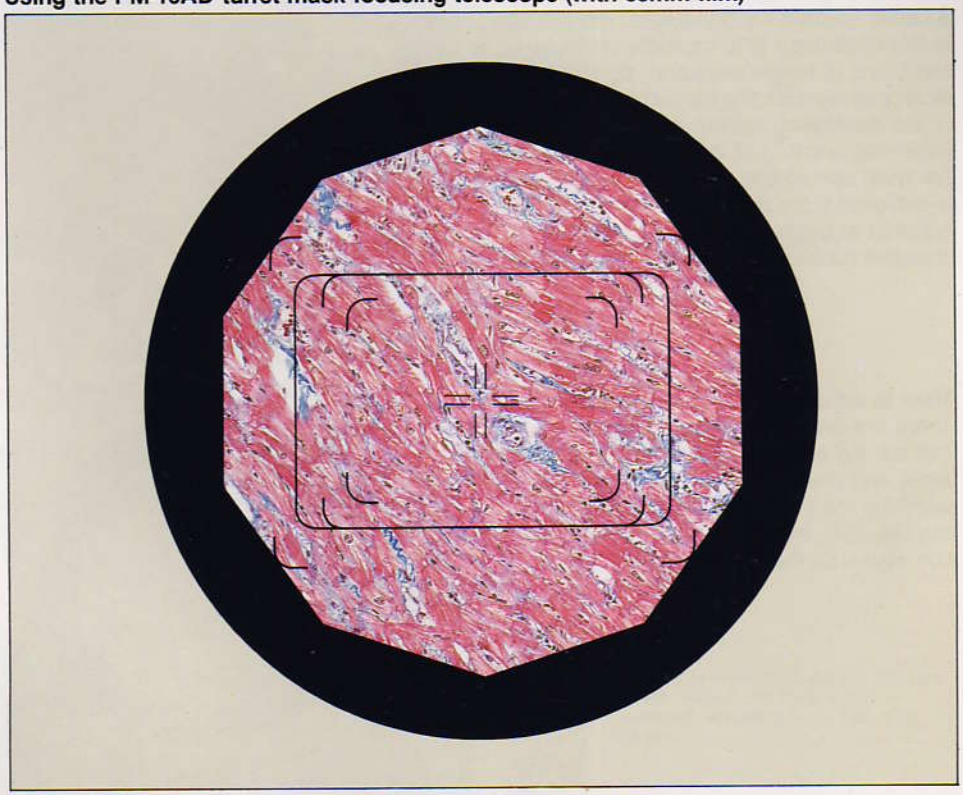


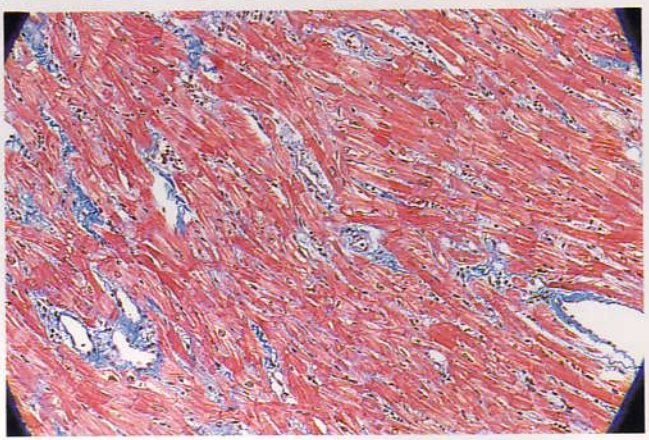
Use of the field iris diaphragm

The field iris diaphragm serves to adjust the illuminated area on the specimen depending on the objective power. This diaphragm plays a crucial role during photomicrography, and if opened wider than necessary, illuminating light is reflected and scattered irregularly on the specimen, resulting in a loss of image contrast. Stopping down the field diaphragm to just beyond the frame reticle area will result in photographic images with improved contrast.

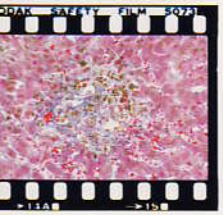
- If the field iris diaphragm is stopped down too close to the frame reticle, the photographed image may be cut at the corners. The diaphragm should therefore be opened slightly more than the reticle shows.

Using the PM-10AD turret mask focusing telescope (with 35mm film)





The corners are cut because the field iris diaphragm has been stopped down too much.

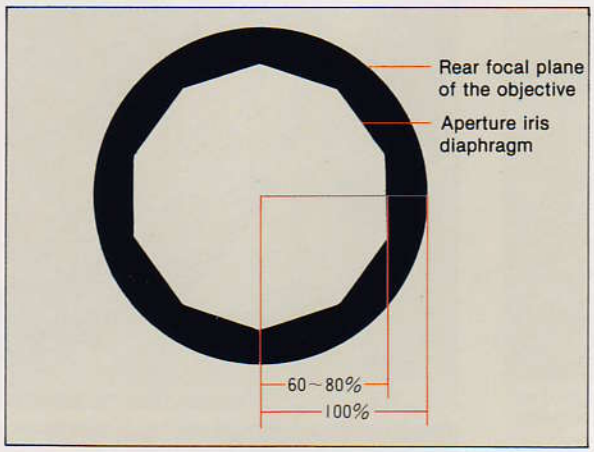


Use of the aperture iris diaphragm and its effects

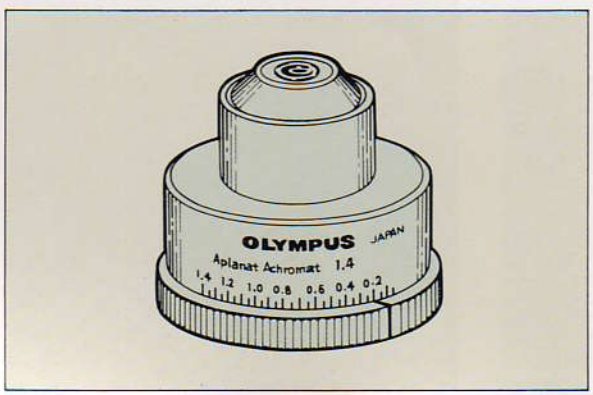
The diaphragm mounted on the condenser is called aperture iris diaphragm. The function of this diaphragm is to maintain optimum conditions of image resolution, contrast and focal depth by adjusting the numerical aperture of the illumination system depending on the numerical aperture of the objective in use. For most specimens optimum image quality is achieved if the aperture diaphragm is adjusted to between 60% and 80% of the objective numerical aperture.

(1)Direct viewing, with the eyepiece removed.

How to adjust the aperture iris diaphragm
There are two methods of adjustment:
Pull out the eyepiece with the specimen in focus and then adjust the diaphragm while watching the iris at the rear focal plane of the objective, as in photo (1); use the graduation marks on the condenser, as in (2).



Reducing the aperture iris diaphragm to 60-80% of the numerical aperture of the objective.



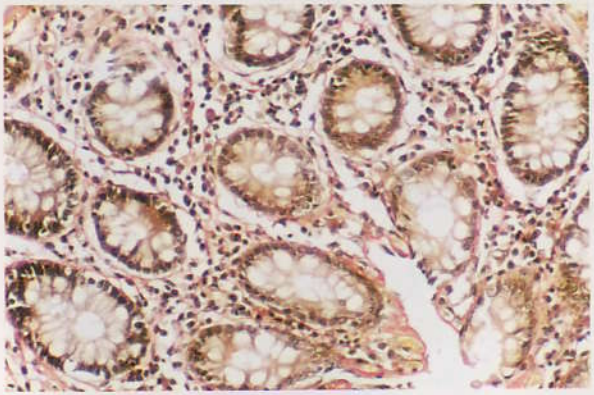
(2)Using the graduation marks on the condenser
Example: Using a 10X objective with a numerical aperture of 0.25 and reducing it to 80%, the graduation mark on the condenser should be set at 0.2(= 0.25 × 0.8).

Effects of the aperture iris diaphragm

Image resolution deteriorates if the aperture iris diaphragm is stopped down too much. With the exception of specially stained thin specimens the diaphragm should not be stopped down lower than to 60% of the numerical aperture of the objective.

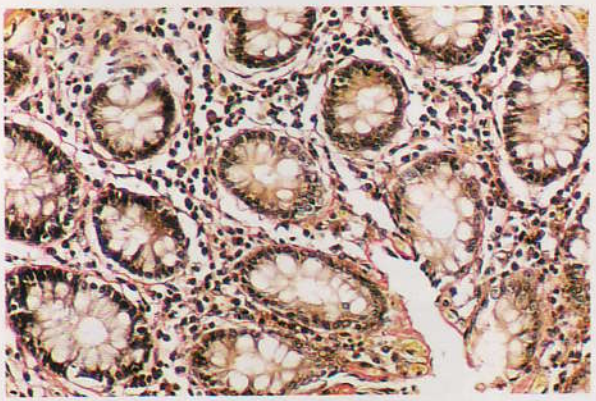
- An effect similar to stopping down the aperture iris diaphragm can be achieved by moving the condenser downward, but this tends to interfere with the basic illumination function of the condenser and results in uneven illumination. Thus always use the diaphragm and do not move the condenser.

Example: When using S Plan Apo 20X, NFK 2.5X



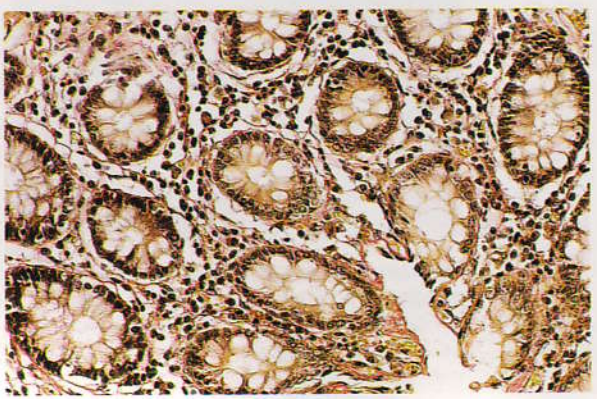
Fully opened position

Overall contrast is low.



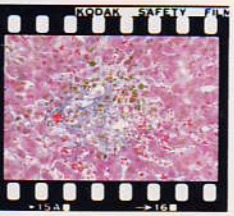
80%

Contrast is enhanced. Details are also clearly visible, and focal depth is increased, resulting in an optimum image.



30%

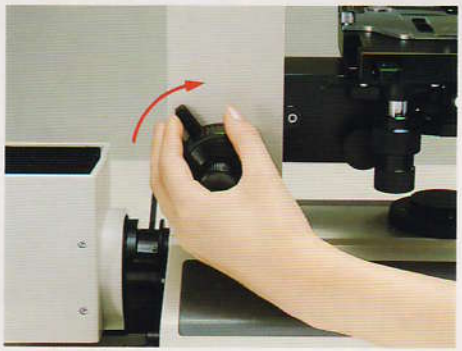
Resolution deteriorates as a result of diffraction.



Basic focusing methods

Focusing for observation
A 10X objective is used as the standard for focusing, then the objective is changed from 10X to 4X and from 10X to 40X and further to 100X. Do not change abruptly from low magnification (2X and 4X) to high (40X and 100X). As a result of the limited eye of the observer and the large focal depth of low magnification objectives (2X and 4X), the front mount of the high-magnification objective could touch the specimen surface when the revolving nosepiece is rotated.

1

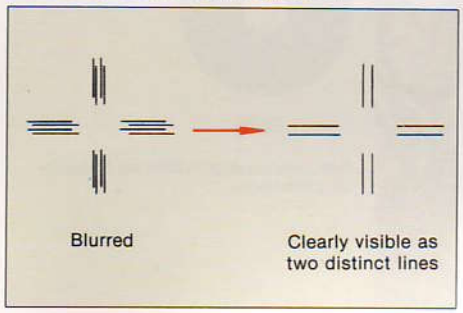


After focusing on the specimen with 10X objective, set the upper limit of the coarse adjustment excursion with the prefocusing lever.

2

Move the specimen detail to be examined to the center of the visual field and increase magnification by rotating the nosepiece.

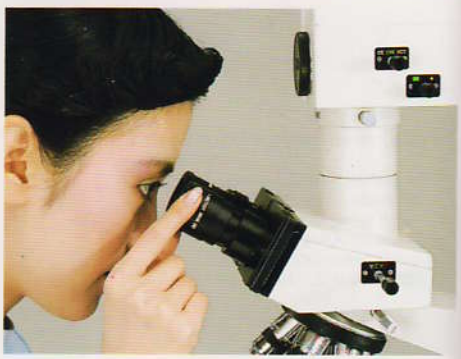
Focusing for photomicrography
Focusing during photography is done either through the focusing telescope of the photographic attachment or through the eyepieces of the binocular tube. When focusing through the eyepieces, a finder eyepiece must be used. Prior to photomicrography the finder eyepiece has to be focused, by means of its focusing front lens, to make clearly visible double cross lines as two parallel lines in the center of the framing reticle.



1

Focusing telescope of the photographic attachment
For photography the specimen focus must be adjusted with the same eye with which the cross lines were focused.

2



Finder eyepiece of the binocular tube
Focusing through the binocular tube is possible with microscopes Vanox, BHS, BHT, and BHTU.

3

If the image is slightly out of focus after change of magnification, use only the fine-focusing knob for refocusing. In particular, do not use the coarse-focusing knob for focusing a high-magnification objective, since there is a danger that the objective will run into the specimen and get damaged.

3

The finder eyepiece is provided with a locating pin. The pin must be firmly inserted into the groove of the eyepiece sleeve. If the pin is improperly inserted, correct focusing becomes impossible, and the image will be out of focus.

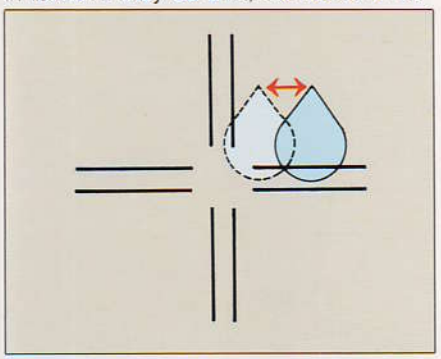
1. Focusing when using 1X, 2X, and 4X objectives

Focusing errors occur quite frequently with low-magnification objectives. Therefore use a focusing magnifier and adjust the focus by following the procedures listed below. But before that the photographer should adjust diopter at the cross lines of the focusing telescope or the finder eyepiece. *(refer to page 21)*

Clamp the focusing magnifier on the focusing telescope, and slide the top section in or out, thereby readjusting the focus at the cross lines. Focus is correct if both the cross lines and the specimen image are clearly visible simultaneously.

2. Focusing when using 10X and 20X objectives

Focusing is achieved by adjusting the cross lines so that they are clearly visible, and then rotating the fine-focusing knob until both the cross lines and the image of the specimen are clearly visible simultaneously. By slightly moving your eyes to all sides, see if the positions of the cross lines and the specimen image do not shift. If this is the case the picture is in focus. If they do shift, the focus is not



properly aligned and must be readjusted with the fine-focusing knob.

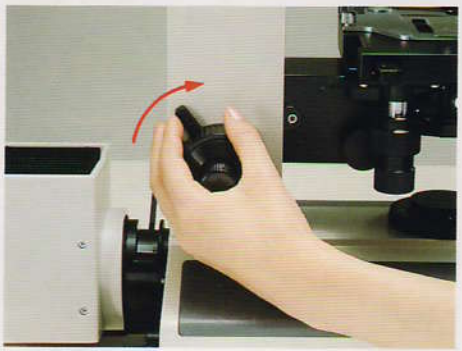
3. Focusing when using 40X and 100X objectives

Adjust the cross lines so that they are clearly visible, then slowly adjust the focus of the specimen image with the fine-focusing knob until cross lines and image of the specimen are clearly visible simultaneously.

Basic focusing methods

Focusing for observation
A 10X objective is used as the standard for focusing, then the objective is changed from 10X to 4X and from 10X to 40X and further to 100X. Do not change abruptly from low magnification (2X and 4X) to high (40X and 100X). As a result of the limited eye of the observer and the large focal depth of low magnification objectives (2X and 4X), the front mount of the high-magnification objective could touch the specimen surface when the revolving nosepiece is rotated.

1



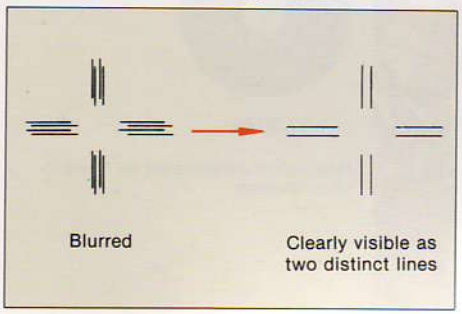
After focusing on the specimen with 10X objective, set the upper limit of the coarse adjustment excursion with the prefocusing lever.

2

Move the specimen detail to be examined to the center of the visual field and increase magnification by rotating the nosepiece.

3

Focusing for photomicrography
Focusing during photography is done either through the focusing telescope of the photographic attachment or through the eyepieces of the binocular tube. When focusing through the eyepieces, a finder eyepiece must be used. Prior to photomicrography the finder eyepiece has to be focused, by means of its focusing front lens, to make clearly visible double cross lines as two parallel lines in the center of the framing reticle.



1

Focusing telescope of the photographic attachment
For photography the specimen focus must be adjusted with the same eye with which the cross lines were focused.

2

Finder eyepiece of the binocular tube
Focusing through the binocular tube is possible with microscopes Vanox, BHS, BHT, and BHTU.

3

3

If the image is slightly out of focus after change of magnification, use only the fine-focusing knob for refocusing. In particular, do not use the coarse-focusing knob for focusing a high-magnification objective, since there is a danger that the objective will run into the specimen and get damaged.

3

The finder eyepiece is provided with a locating pin. The pin must be firmly inserted into the groove of the eyepiece sleeve. If the pin is improperly inserted, correct focusing becomes impossible, and the image will be out of focus.

1. Focusing when using 1X, 2X, and 4X objectives

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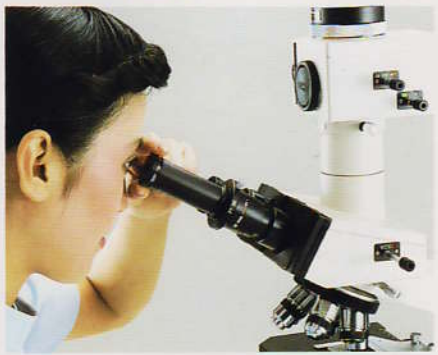
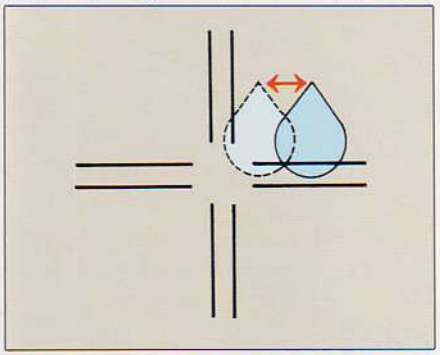


Clamp the focusing magnifier on the focusing telescope, and slide the top section in or out, thereby readjusting the focus at the cross lines. Focus is correct if both the cross lines and the specimen image are clearly visible simultaneously.

2. Focusing when using 10X and 20X objectives

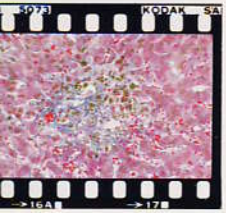
Focusing is achieved by adjusting the cross lines so that they are clearly visible, and then rotating the fine-focusing knob until both the cross lines and the image of the specimen are clearly visible simultaneously. By slightly moving your eyes to all sides, see if the positions of the cross lines and the specimen image do not shift. If this is the case the picture is in focus. If they do shift, the focus is not



properly aligned and must be readjusted with the fine-focusing knob.

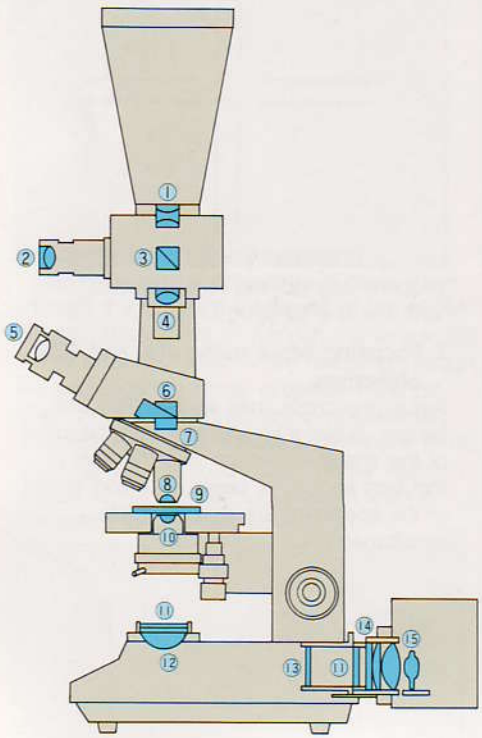
3. Focusing when using 40X and 100X objectives

Adjust the cross lines so that they are clearly visible, then slowly adjust the focus of the specimen image with the fine-focusing knob until cross lines and image of the specimen are clearly visible simultaneously.



How to spot dirt and specks of dust in the optical systems of the microscope and the photomicrographic attachment

Dirt and dust particles are sometimes noted during observation or photogrpahy, but pinpointing their exact location may be difficult. When photographing important specimens which cannot be photographed again it can be really frustrating if dust particles are visible on the picture. An effective method is therefore required that will help you detect contamination.



■ Since dirt at locations no. ① and ③ may come from particles of the film itself (loading, film transport), regular checks are necessary.

■ If the aperture diaphragm is reduced below 30%, dirt and dust specks become clearly visible.

■ Dust specks on the prism inside the observation tube, on the inside of the objective, and on the inside of the microscope base cannot be wiped off easily. If cleaning becomes necessary, contact your Olympus dealer for service.

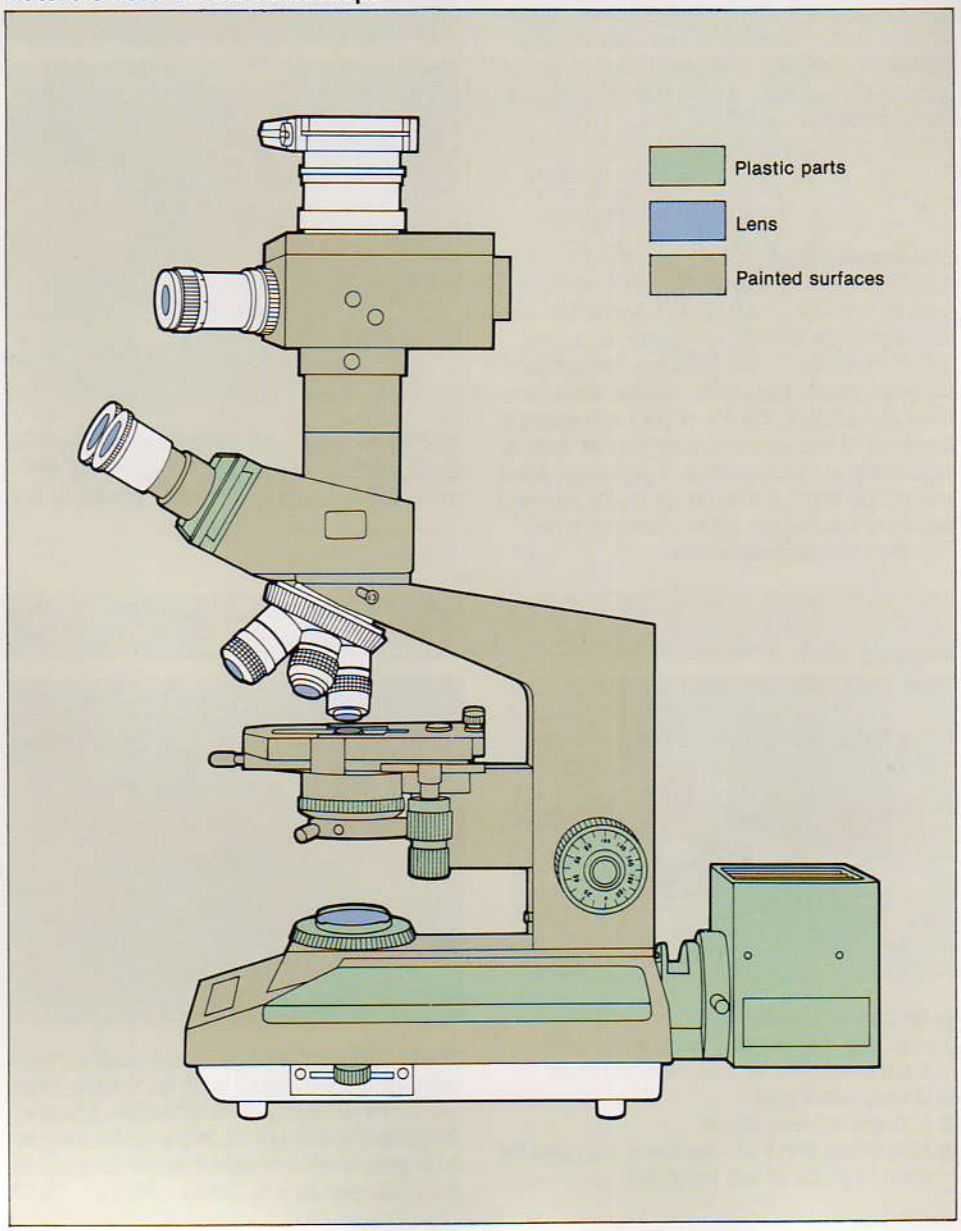
Location	Description	Observation	Photography	Method of verification
①	Relay lens for large-format camera		○	Remove the adapter for large-format cameras and check for dirt by peering in through the top. If you spot dirt, unscrew and remove the relay lens and clean it.
②	Focusing telescope	○		Rotate the top lens element as you observe the image.
③	Camera prism		○	Set to Time mode, open the shutter, and peer in through the top.
④	Photo eyepiece		○	Either remove photo eyepiece and check for dust particles or leave photo eyepiece in place, rotate it and check for moving dust particles.
⑤	Eyepiece	○		Rotate the eyepiece as you observe the image.
⑥	Optical path selector prism	○	○	Change the optical path while alternately observing through the focusing telescope or the finder eyepiece.
⑦	Tube length correction prism	○	○	Remove the observation tube from the microscope body and check the prism surface for fingerprints or contamination.
⑧	Objective	○	○	Remove the objective from the nosepiece and check it for dirt or contamination.
⑨	Specimen	○	○	Observe the specimen and move it in the field. If dust is on the specimen, it will also move.
⑩	Condenser	○	○	Remove the condenser from the microscope and look for dirt and oil deposits.
⑪	Filter	○	○	Check the filter after removing it from the microscope base.
⑫	Light exit window	○	○	Switch on the illumination and examine the lens at an angle.
⑬	Frosted glass	○	○	Remove the lamp housing from the base, and check the frosted glass.
⑭	Collector lens	○	○	Remove the lamp housing and check the lenses in the collector assembly.
⑮	Bulb	○	○	Remove the bulb from its socket and check for signs of blackening, fingerprints, dirt, etc.

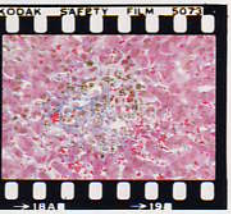
How to clean the microscope frame

Stains on the microscope frame are first wiped with a piece of cloth wetted with a small amount of neutral detergent, and then wiped clean with a piece of cloth that has been immersed in luke-warm water. But make sure not to touch the lens section while cleaning the microscope frame.
Avoid using organic solvents which may damage plastic parts.

For dust contamination that adheres to painted parts and is difficult to remove, wipe with a piece of cloth or soft tissue paper that had been soaked in a mixture of 7 parts ether and 3 parts alcohol. Keep the mixture away from plastic parts to prevent damage.

Materials used for the microscope





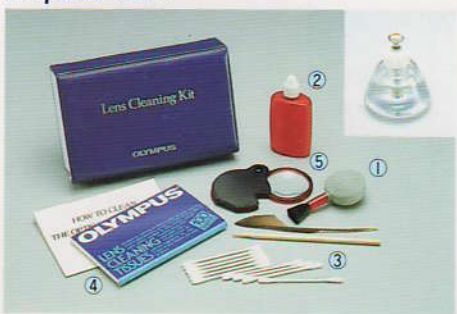
How to clean the optical system

Keeping the optical system clean at all times is most essential. But if dust gets onto a lens surface, it can normally be removed with a blower. You should, however, make a habit of covering the microscope with a dustproof cover, after each use.

Cleaning method

If dust spots on optical glasses such as lenses, prisms, and filters are left unattended, the dust becomes difficult to remove and may cause mold. By always keeping optical glass surfaces clean, you avoid maintenance problems and prolong the life of your microscope. Cleaning of the lens surfaces applies only to exposed areas of objectives, eyepieces, filters and condensers. If internal or major cleaning becomes necessary, please contact your Olympus Microscope dealer.

Required tools

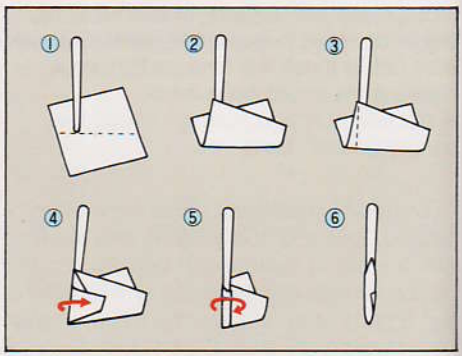


① Air gun or blower brush
② Cleaning mixture of 7 parts ether and 3 parts alcohol, or lens cleaning fluid.
③ Q-tips, wood stick
④ Soft gauze, lens tissue
⑤ Magnifying glass. An eyepiece can also be used in place of the magnifier.

1

To prevent scratches on coatings and optical glass, remove dirt and dust that sticks to their surfaces with an air gun or blower brush.

2

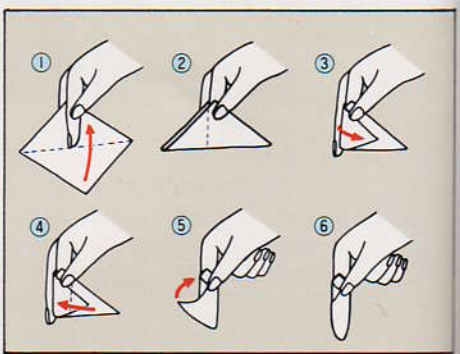


Wrap the lens tissue around a wooden or bamboo stick as illustrated.

5

When cleaning large glass surfaces on both sides of an accessory such as a filter, fold two or three layers of lens tissue soaked in the cleaning mixture, hold the accessory at its edges, and wipe from the center towards the periphery as you slowly rotate it.

6



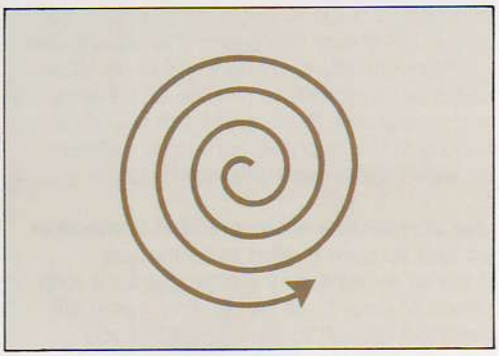
When cleaning the surfaces of the condenser and of the light exit glass hold a piece of lens tissue between your middle and index fingers, fold it and wrap it around your index finger. Then hold the tissue down with your thumb while wiping the lens surfaces clean.

3

Clean the lens by putting a small amount of lens cleaning fluid or cleaning mixture on the tip of a lens tissue. Discard each lens tissue after a single use.

4

When cleaning a large lens surface, wipe from the center towards the periphery in a circular motion.



7

After cleaning, examine the lens surface with a magnifying glass. If color reflected from the lens surface looks uneven, it is an indication that there are still dust specks and dirt on the lens.

By viewing through the bottom of an eyepiece, you can use it as a magnifying glass.

8

When cleaning a large lens with lens tissue wrapped around your finger, you should wipe from the center towards the periphery in a circular motion. Always, use a clean portion of the lens tissue as you rotate your index finger.

After cleaning, breathe lightly on the lens, surface until the whole surface has turned white, then check whether the haze disappears uniformly. Spots where the haze disappears only slowly are not yet wiped clean.

How to clean an oil-immersion objective

Clean the oil-immersion objective during examination

After finishing observation with an oil-immersion objective, wet a pad of cotton-wool or a piece of lens tissue with a small amount of cleaning mixture containing 7 parts ether and 3 parts alcohol, to remove oil adhering to the objective. Since an oil film will often adhere to the objective front lens, it should be wiped clean twice after each use.

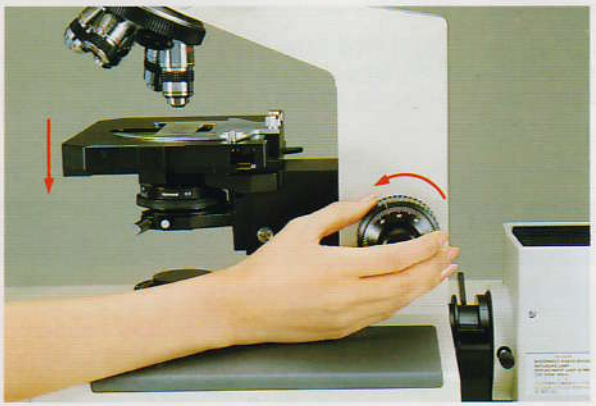
Use only factory recommended immersion oil and remove it after examination

If the oil remains on the objective for a long period of time, it will harden (e.g. cedar oil), making it difficult to remove even if you wipe the lens repeatedly. The lens may be damaged in the process. Use only specified oil, and after use, wipe the oil from the immersion surface of the objective, keeping it clean at all times.

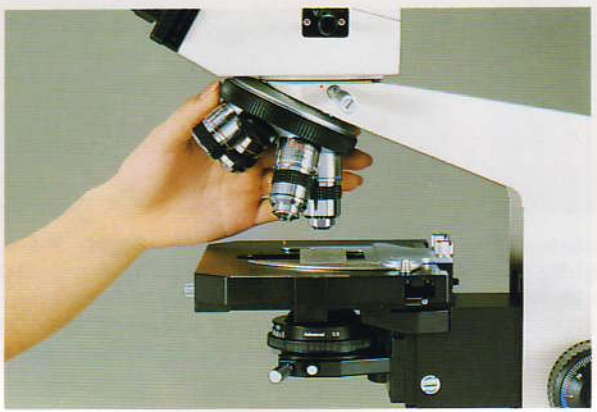
If you frequently use an oil-immersion objective, oil may contaminate the surface of a dry-type objective when you change objectives. To prevent the oil from adhering to the objective, carefully rotate the nosepiece after lowering the stage, so that the oil does not touch any objective.

*If the image of a dry high-magnification objective appears fuzzy, check for oil that might have adhered to the tip of the objective.

Wipe the front lens of the objective twi



Lower the stage slowly so that no oil w
touch the front lens of the dry objectiv



After lowering the stage, carefully rota
nosepiece and click the dry objective i
optical path.

How to clean specimens

Make it a habit to clean each specimen both before and after observation. Otherwise, dirt and dust that you failed to notice during observation might appear on the photo. For cleaning the specimen a soft cloth, gauze, or piece of lens tissue may be used without cleaning liquid. But if the contamination is difficult to clean, breathe on the specimen before wiping it. When cleaning the specimen, wipe both surfaces.

Points to note during cleaning

(1) Removal of oil as well as routine cleaning can be done more easily if the specimen is removed from the stage.
(2) When using cleaning mixture or lens cleaning fluid, use a moistened cloth or cleaning tissue. Be certain not to apply excessive fluid, as it may seep underneath the cover glass and damage the specimen.

There are two types of specimens: those with a cover glass, and those without a cover glass, such as blood smears.

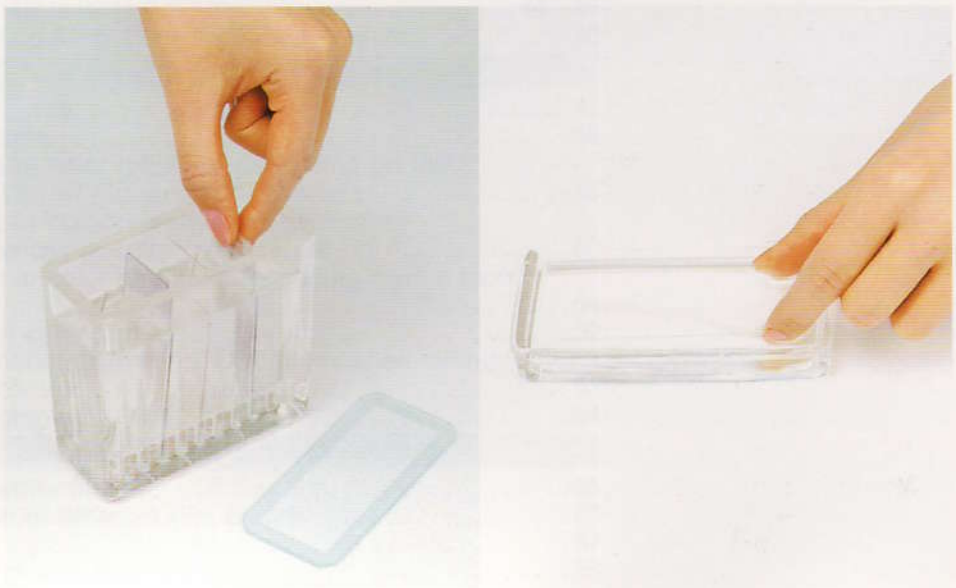
1. Cleaning specimens with cover glasses

Just as when cleaning lenses, wipe off the oil and dirt with a piece of lens tissue lightly moistened with cleaning mixture. Because the oil cannot be completely removed with one wipe, repeat wiping until the oil film is removed.

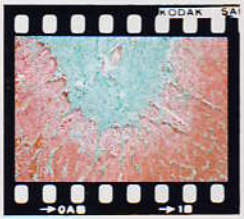
2. Cleaning specimens without cover glasses

Oil adhering to uncovered specimens cannot be wiped off. You can, however, remove the oil by immersing the specimen for 5 to 10 minutes in a xylene bath. There are containers for both horizontal and vertical immersion, the proper selection of which depends on your particular need.

Cleaning specimens with cover glass

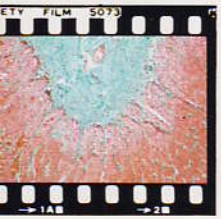


Cleaning specimens without cover glass



Section 2

When taking pictures in photomicrography



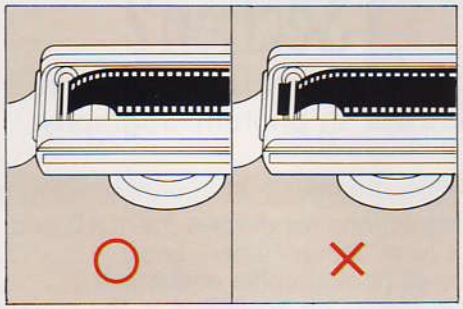
Basic information in photomicrography

Operating Instructions for models PM-10AD and PM-10ADS (with 35mm camera back)

1 Setting the film format

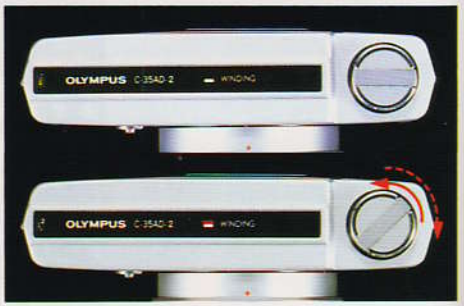
Push the button for 35mm camera.

2 Load the film properly



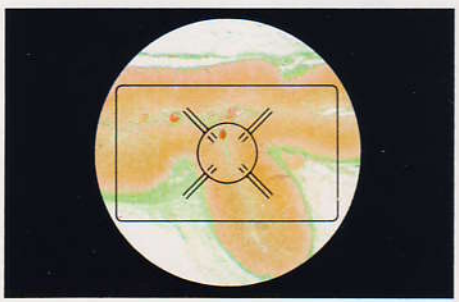
Load the film into the camera and make sure that the end of the film does not protrude beyond the spool groove. Otherwise the frame spacing may not be uniform.

3 Confirm the winding of the film



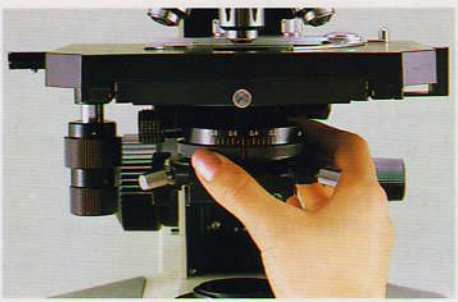
Wind the film onto the empty take-up spool and advance it to frame no. 1. To confirm that the film is winding, be certain that the film rewinding crank is rotating. If it is not, rotate the crank 2 or 3 times in the direction of the dotted arrow to pick up the slack of the film inside the camera, and again check the rewinding crank to confirm the winding of the film.

7 Center the specimen to be photographed in the visual field



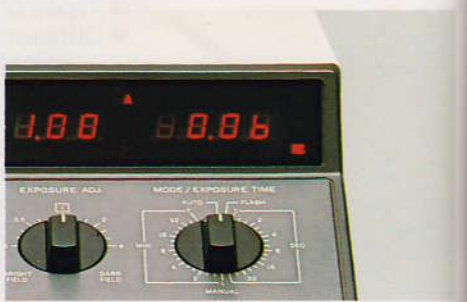
Move the area of the specimen you want to photograph into the center of the visual field with the stage controls. When you use model PM-10ADS, center the area to be photographed in the spot metering section of the viewfinder.

8 Focus and adjust the aperture iris diaphragm



Focus on the specimen and adjust the condenser aperture iris diaphragm so that suitable contrast is achieved. The aperture diaphragm is ordinarily set at 60% to 80% of the objective numerical aperture. *(refer to pages 24-25)*

9 Adjust shutter speed



Check the shutter speed. Adjust the speed to between 0.01 and 0.5 seconds by using ND filters.

4 Set film speed

Set the speed for the film used.

5 Set the characteristics for reciprocity failure

Set the characteristics for reciprocity failure for the film used.

6 Measure color temperature (for color photography) Set the voltage above 6V. (for black-and-white photography)

Color photography

Set the color temperature to match the type of color film used. Measure the temperature at a blank area not covered by the specimen.

Type of film	Light balancing filter	Dial position on CTR
Daylight film	LBD-2N	D
Tungsten film	LBT	T

The voltage position when not using the color temperature meter CTR is:
8.5—9.5V. for models BHS and
4 or 6V. for models BHT and BHTU

Black-and-white photography

Set the voltage above 6V. Normally, a green filter is used.
(For emphasizing particular colors refer to page 57)

10 Exposure adjustment

Exposure adjustment is based on the total distribution of the specimen.*(refer to pages 42-43)*

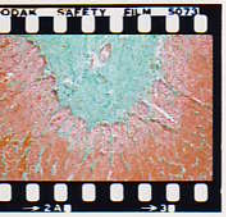
11 Focusing the image

Focus is adjusted either through the focusing telescope of the PM-10AD/PM-10ADS or the binocular tube of the microscope.*(refer to pages 26-27)*

- The focusing telescope of the PM-10ADS/PM-10AD includes different photo frames for different types of film.
- The type of finder eyepiece varies with film size and each type of finder eyepiece includes several photo frames indicating different magnifications of NFK photo eyepieces.

12 Release the shutter

Press the shutter release button. After completing exposure, check for the sound of film winding.

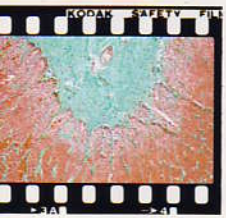


Magnification of the photographic equipment

Photo magnification on the film surface varies with the projection length of the photographic equipment and the type of photo eyepiece used. In all cases photo magnification is computed by multiplying the objective magnification and the photo eyepiece magnification with the coefficient listed in the table on the right.

Photographic equipment \ Photo eyepiece	FK, NFK-type	P-type
PM-10AD PM-10ADS	1 X	0.5 X
PM-10M	1 X	0.5 X
PM-6	0.9X	0.45X
BH2-PM-6	0.8X	0.4 X

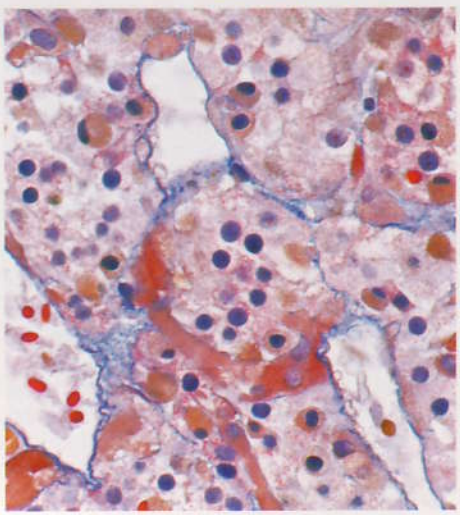
When using models PM-10AD or PM-10ADS; 10X objective and 2.5X NFK photo eyepiece:
Photo manification = 10 × 2.5 = 25



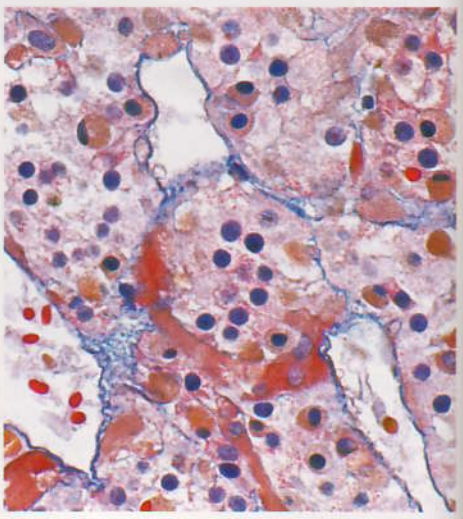
Differences in resolution according to the combinations of objectives and eyepieces

Even though when the overall photo magnification is the same, resolution varies depending on the combination of objective and photo eyepiece. The resolution on the film plane is improved if the magnification of the photo eyepiece is low and that of the objective high. As the photo shows, a combination of objective 40X and NFK 2.5X provides a clear display of even minute image detail. Focal depth, however, becomes shallow.

Example: Photo magnification of 100X



Objective S Plan 20X and photo eyepiece NFK 5X



Objective S Plan 40X and photo eyepiece NFK 2.5X

Setting of photographic magnification (effective magnification)

When taking pictures, you have to first decide on the desired enlargement ratio. The magnification on the film plane is generally determined by objective magnification times photo eyepiece magnification. But the effective magnification of the picture changes with the numerical aperture of the objective, and is normally based on the following relationship, which must be taken into account when enlarging the photomicrograph.

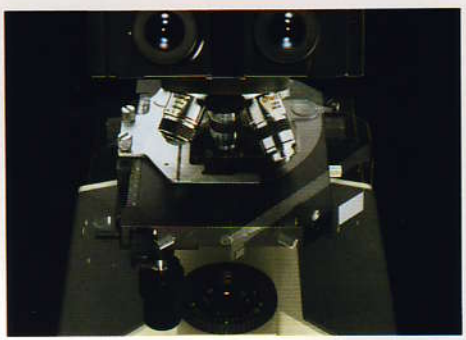
*The effective magnification is based on the assumption that the picture is viewed within the closest distance affording distinct vision. The values listed below do not apply when trying to project 35mm format slides.

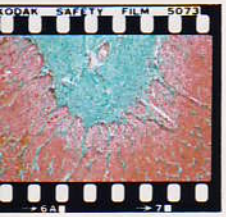
$500N.A. < M < 1000N.A.$ *N.A. = numerical aperture of the objective M = effective magnification*

Objective magnification	4X		10X		20X		40X		100X	
Type of objective	S Plan Achromat	S Plan Apochromat	S Plan Achromat	S Plan Apochromat	S Plan Achromat	S Plan Apochromat	S Plan Achromat	S Plan Apochromat	S Plan Achromat	S Plan Apochromat
N.A.	0.13	0.16	0.30	0.40	0.46	0.70	0.70	0.95	1.25	1.30
Effective magnification	65～130	80～160	150～300	200～400	230～460	350～700	350～700	475～950	625～1250	650～1300

Framing of the specimen

During photography, the specimen often does not fit into the photo frame. This problem can be overcome by rotating the camera section in relation to the specimen, though operation of the microscope becomes more difficult. Here, the framing of the specimen is carried out the preferred way, by rotating the stage.





Checking of the finished photomicrographs

Photomicrography involves scientific photography, which makes it imperative that the photographer accurately records his findings on film. Additionally, the photograph should convey a strong esthetic impression to the viewer. Valuable records should not be documented with run-of-the-mill photos.

To further improve your results in photomicrography, it is important that you always check your own photos. By referring to the following checkpoints, you can pinpoint any problems concerning your photos. Then proceed to the second half of this section titled Photomicrography Techniques.

1. Questions relating to all photography

(1) Is the image in focus *(refer to pages 26-27)* and the exposure properly adjusted? *(refer to pages 42-46)*

(2) Have dirt or dust specks on the specimen found their way onto the photograph? *(refer to pages 28-33)*

(3) Is the specimen properly stained?

(4) Is there uneven illumination? *(refer to pages 22-25)*

2. Color photography *(refer to pages 47-54)*

(1) Is the background (empty space) white or of light grey tone?

(2) Is the color of the specimen accurately reproduced?

3. Black-and-white photography *(refer to pages 55-57)*

(1) Does the finished photograph show clearly graded black and white tones without excessively dark (solid black) shadow areas and washed-out (completely white) highlights?

Purpose **Film** Kodachrome 25 **Date** 1984 Dec. 1

No.	Objective	Photo eyepiece	Specimen	Filter	Voltage	Aperture iris diaphragm	Exposure adjustment	Remarks
1	S Plan 10X	NFK 2.5X	KIDNEY	LBD	10V	FULL	1 X	
2	〃	〃	〃	〃	〃	70%	〃	
3	〃	〃	〃	LBD+CC05M	〃	FULL	〃	
4	〃	〃	〃	〃	〃	70%	〃	
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								

Purpose _____ Film _____ Date _____

No.	Objective	Photo eyepiece	Specimen	Filter	Voltage	Aperture iris diaphragm	Exposure adjustment	Remarks
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
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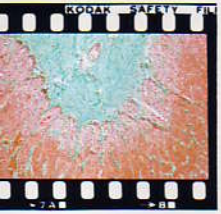
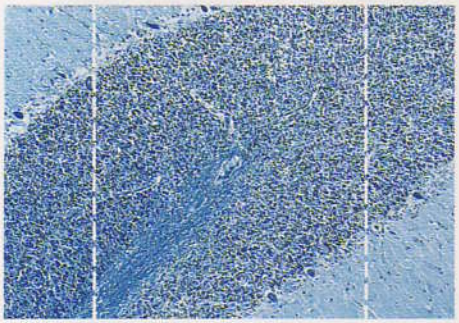


Photomicrography techniques

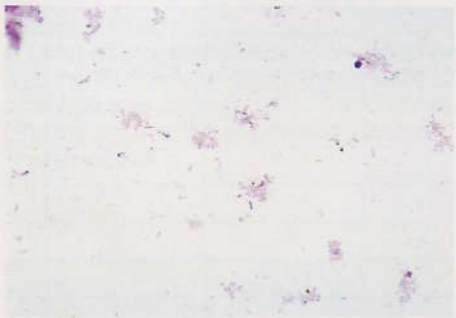
Exposure adjustment techniques

When taking photomicrographs, exposure compensation is necessary depending on the distribution of the specimen in the field. But if the specimen is evenly distributed within the integrated or spot metering range, no compensation is required (in case of 1X).

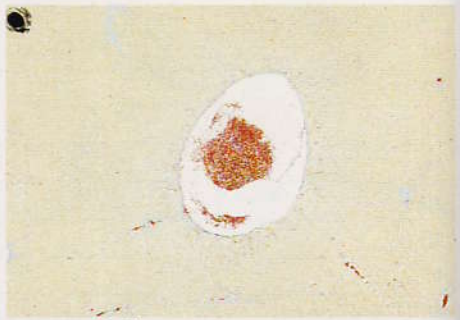
1. PM-10AD
(60% Average metering with model PM-10AD)



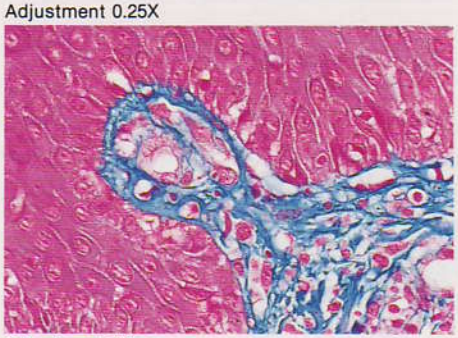
Average metering 60%



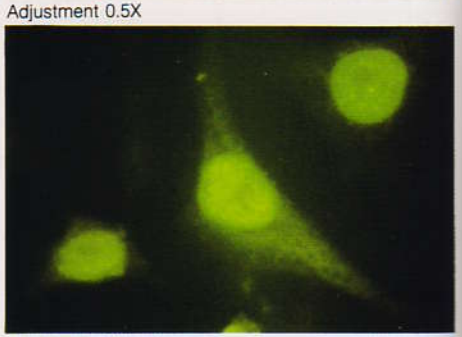
Adjustment 0.25X



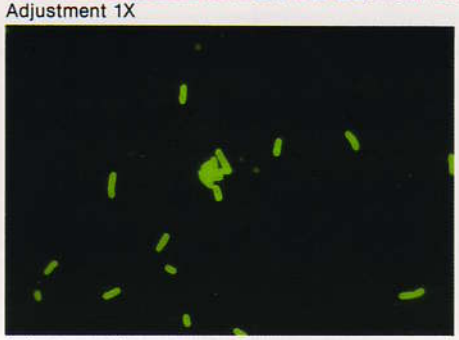
Adjustment 0.5X



Adjustment 1X

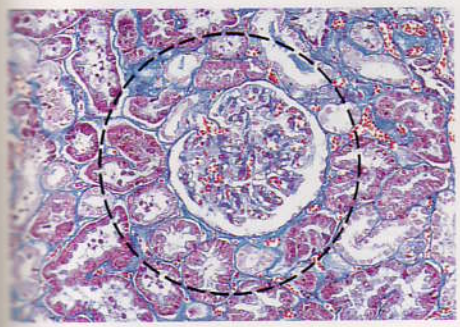


Adjustment 2X



Adjustment 4X

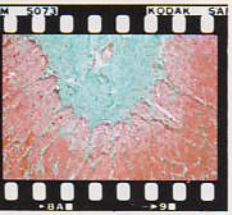
2. PM-10ADS
(Model PM-10ADS can be used for integrated metering of 30% and spot metering of 1%.)



Integrated metering 30%

Spot metering 1%

Measuring area	Specimen condition within the 1% metering area	Exposure adjustment dial setting	Reciprocity failure characteristics
	Brightfield background dotted with fairly dense specimens	0.25X	Set the reciprocity failures compensation value for the film being used.
	Brightfield background containing scattered specimens	0.5X	
	Specimen is evenly distributed within the 1% metering area.	1X	
	About half (50%) of the specimen is distributed within the dark background	2X	
	About one-fourth (25%) of the specimen is distributed in the dark background	4X	
	The dark background is dotted with specimens	Joint use of the ISO/ASA sensitivity dial	



Use of the AE lock

By pressing this button during the automatic exposure mode, you can adjust the exposure time indicated on the display panel (expected exposure time, actual exposure time).

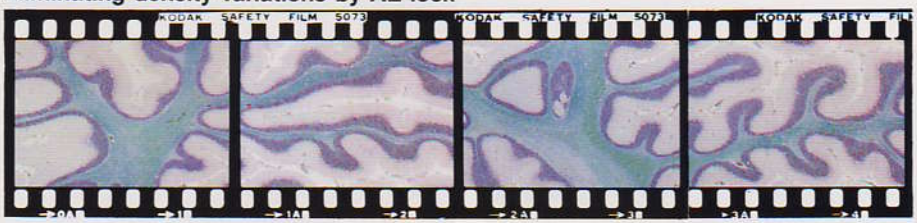
1. Taking panoramic pictures using the AE lock

For panorama pictures where any number of copies is taken from different sections of the same specimen or where several photos are patched together, perfect panoramic photographs with uniform density can be taken by locking-in a fixed exposure for all the photomicrographs.

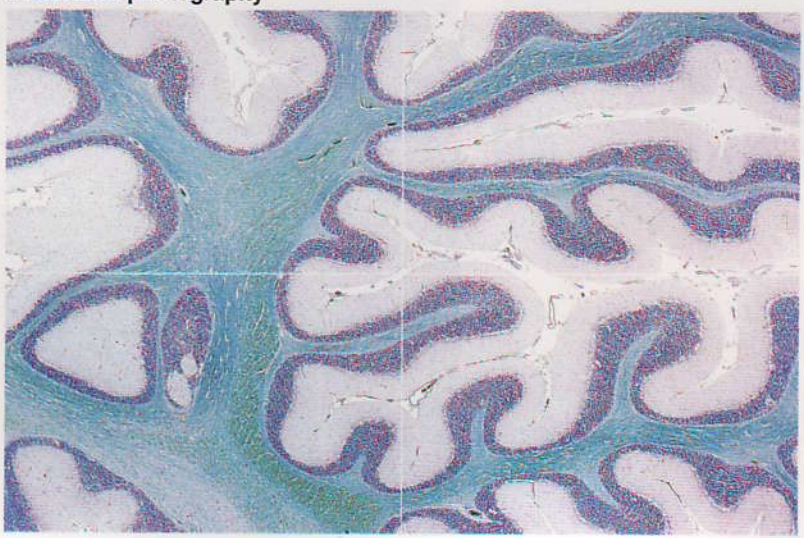
AE lock operation

PM-10ADS (integrated metering 30%, spot metering 1%)	PM-10AD (average metering 60%)
When pressing the AE lock button, the warning light above it flashes to indicate AE lock mode. The unit is now ready for photographing. To cancel this function press the AE lock button again. This switches the light off, and the device reverts to normal automatic exposure mode.	After positioning the specimen to be photographed and pressing the AE lock button, the warning light above it flashes to indicate AE lock mode. Then, after taking the first photograph, exposure time is locked-in. To cancel this function press the AE lock button again. This switches the warning light off, and the device reverts to the normal automatic exposure mode.

Eliminating density variations by AE lock



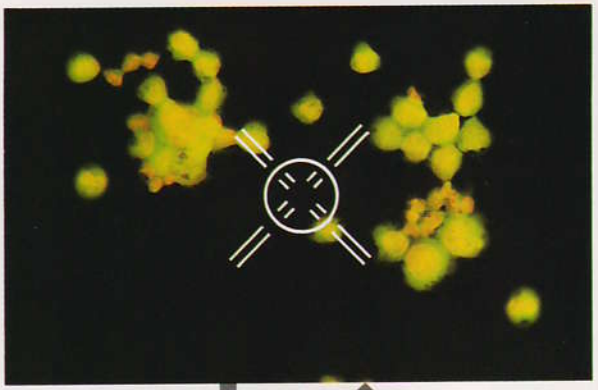
Panorama photography



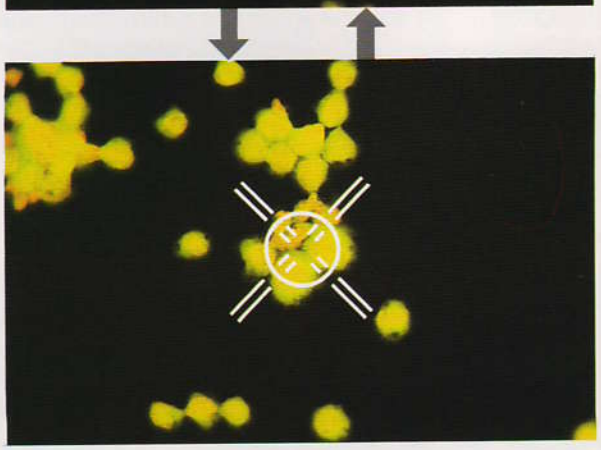
2. Example of using the AE lock on model PM-10ADS

(1)In combination with spot metering

If specimen areas requiring spot metering are not centered because of framing problems, they are first positioned in the center and the AE lock is activated. Then the specimens are moved back to their original position and photographed.



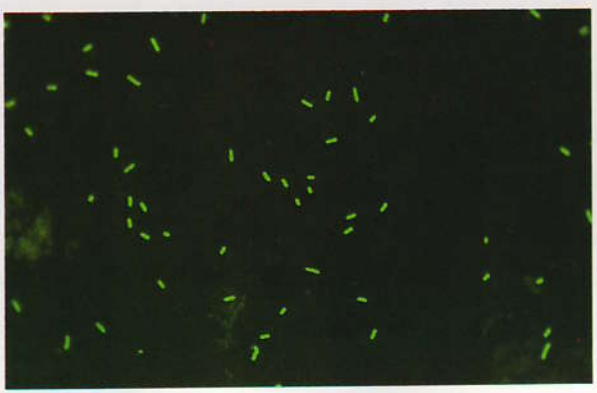
1 The specimen detail is at the location where it is to be photographed, but exposure cannot be measured.



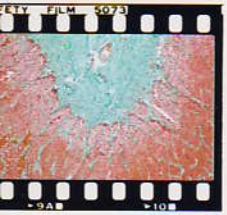
2 After moving the specimen detail to the measuring area and activating the AE lock, the specimen detail can now be moved back to its original position and the photograph is taken.

(2)Photographing within a range close to maximum exposure time

If exposure display is close to the maximum exposure time, for example during fluorescence photomicrography, the photographic equipment might display an underexposure warning during automatic exposure. This can be prevented by using the AE lock, resulting in long-time exposure.



The SAFETY lamp comes to red and an audible warning sounds.



Compensating for a film's reciprocity failure

With normally used photographic emulsions there is a rule (the reciprocity law) that determines the luminance of the light striking the film surface. According to this rule, the total amount of exposure is defined as the product of the luminance and the exposure time. For example, the amount of exposure with 1/60 sec. exposure at f8 is the same as for 1/30 sec. at f11. But for longer exposure times this rule no longer applies, leading to under exposure and changes in color reproduction. This phenomenon is known as reciprocity law failure. But since, in photomicrography, exposure compensation cannot be carried out via the aperture diaphragm, exposure time is lengthened or shortened to obtain a suitable exposure level. If the reciprocity dial is set on models PM-10ADS and PM-10AD, compensation is carried out automatically, resulting in proper exposure. Uneven color reproduction must be compensated for with a CC filter.

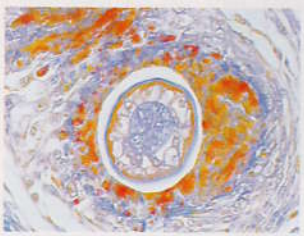
The above chart lists the compensation data for reciprocity law failure characteristics when using Kodachrome 25 film for general photomicrography. The chart shows that for long exposure times exceeding 1 sec., both exposure time compensation and color compensation by filter are necessary. For additional data on reciprocity law failure characteristics contact the film manufacturer.

Example: Data for the characteristics of reciprocity law failure (Kodak color film DKD-141)

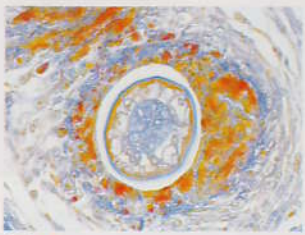
	Exposure time						
Kodachrome 25 ASA 25	1/10,000	1/1,000	1/100	1/10	1	10	100
	No exposure compensation required				+1/2 aperture no filter required	+2 aperture CC10B	+3 aperture CC20B
	No filter required						

Example of compensation (when using Fujichrome RD100)

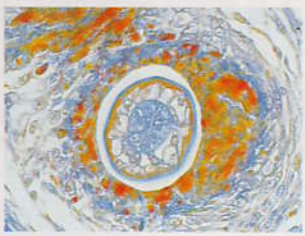
No compensation



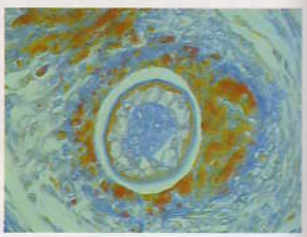
0.02 sec



0.2 sec

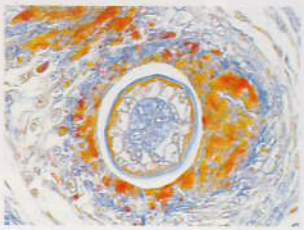


2.4 sec

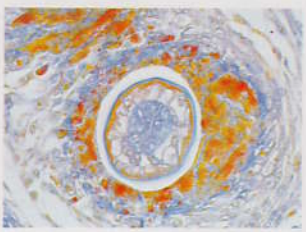


28 sec

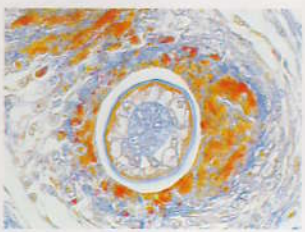
Compensation



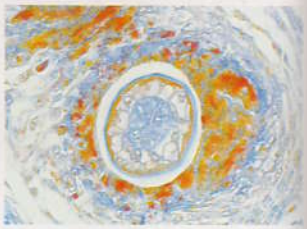
0.02 sec



0.2 sec



3.2 sec



52 sec

Color photomicrography

Color film

Many different types of color film are offered on the market today, leaving the user at a loss as to which brand to use for photomicrography. Normally, daylight-type reversal film with an ISO/ASA speed of 50-100 is used, while microscopes require a light balancing filter (LBD-2, 2N).

Daylight type	Ektachrome 64 Ektachrome 100 Kodachrome 25 Kodachrome 64 Agfachrome CT-18 Agfachrome 50 Type-S Agfachrome 100 Fujichrome 50D Fujichrome 100D
Tungsten type	Ektachrome 50 Kodachrome 40 Agfachrome 50 Type-L

Requirements for the selection of color film

(1) Film with high resolving power, since photomicrography requires display of detailed structures.

(2) The display must be able to discriminate between the fine color differentiations contained in a single specimen.

(3) Faithful reproduction of specimen colors without background discoloration.

In view of these factors, the following conditions can be set for photomicrography:

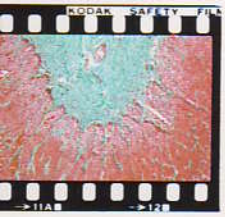
(1) Fine grain

(2) Good color contrast

(3) Good color rendition

For normal photomicrography in transmittedlight bright field illumination color film with an ISO/ASA speed between 50 and 100 will assure satisfactory quality. For special cases such as printed publication or enlargements, Kodachrome film offers good quality.

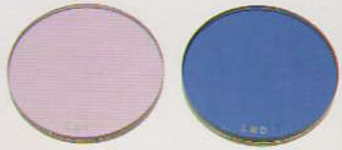
When photographing dark specimens (phase contrast, polarized light, fluorescence), exposure time increases. Although there may be excessive grain, for cases requiring fast shutter speeds use film with a high ISO/ASA speed.



Types of filters

Filters
Selection of filters for use in photomicrography is based on the type of film used. Use LBD-2N for daylight films and LBT filters for tungsten-type film. For changing light intensity, use of a neutral density filter (ND) is recommended. The number on the filter rim gives the transmission value. In case of filter ND6, 94% of the total illuminating light is absorbed and only 6% of the light is transmitted.

Types of filter and their functions
Filters for use in color photomicrography comprise the following main types:

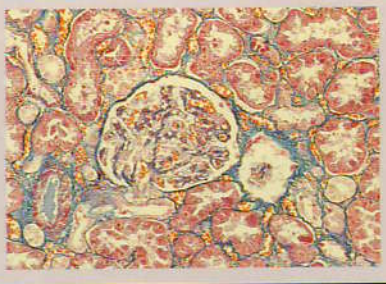
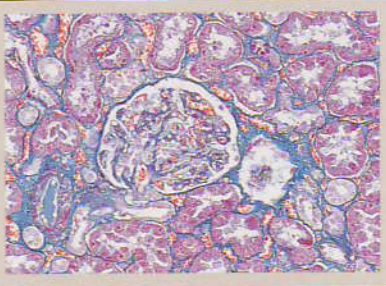
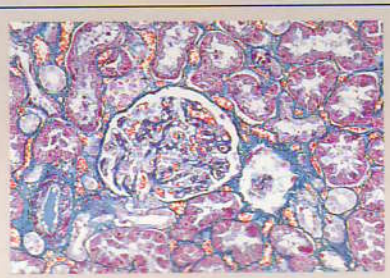
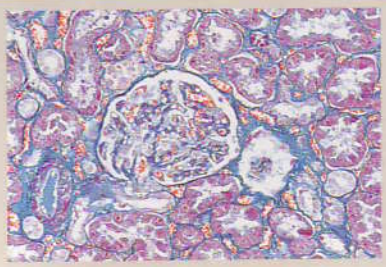
① **Light balancing filter** 	To convert the color temperature of the microscope light source to the film being used. *(refer to page 49)*
② **ND (neutral density) filter** 	To reduce the light intensity without affecting color temperature, when illumination is too bright.
③ **CC (color compensating) filter** 	For color rendition problems such as slight differences in color hue or fading in developed photos. *(refer to page 53)*
④ **Didymium filter (marked FF on Olympus models)** 	To emphasize color, i.e. when trying to compensate for the insufficient intrinsic red color rendition of a film or to enhance the red color of the specimen or the color contrast of Polaroid color film. *(refer to page 58)*
⑤ **Heat absorbing filter** 	To absorb heat rays emanating from the light source of the microscope to prevent damage to or destruction of live specimens. Since this filter transmits a small quantity of blue light, it may become necessary to use a color-compensating filter (CC10Y or CC10M) when taking color photos. *(refer to page 53)*

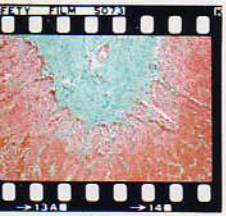
Differences in color rendition depending on the combination of film and light source

There are two types of color films—daylight-type (balanced for sunlight) and tungsten-type (balanced for artificial light), the selection depending upon the light source. Virtually all users have experienced the disappointment of their photos appearing excessively red when they used daylight film under incandescent light conditions.

Similarly, when using the tungsten-type film outdoors, a bluish photograph is obtained. The fault in both cases is failure to use the proper film to match the light source.

Color rendition depending on the combination of film and light source

Type of light source \ Type of film	Daylight-type (sunlight)	Tungsten-type (artificial light)
Sun	 ○	 ×
	 ×	 △
  LBD light balancing filter for daylight-type color film. (sunlight)	 ○	 ○
  LBT light balancing filter for tungsten-type color film. (artificial light)		



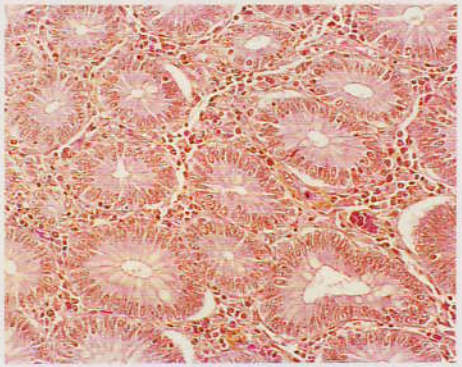
Differences in color reproduction depending on differences in color temperature

Color temperature

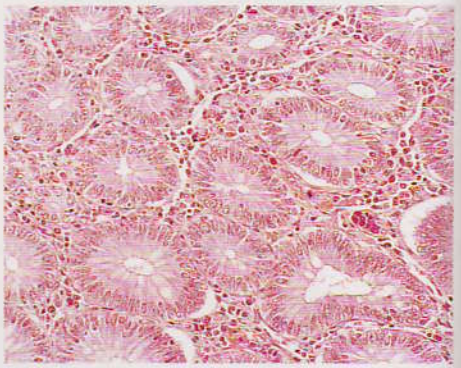
Color temperature designates the properties of the light source. A blackbody radiator, when heated, emanates light of different color depending on the temperature. The properties of the light source can be indicated by referring to the temperature of the blackbody at a fixed temperature level. This feature is called color temperature, the numerical unit being expressed either in absolute temperature or in degrees Kelvin (K).

Color film for use in photomicrography is normally daylight type, but since the light source of the microscope is a tungsten type, its color temperature is low (2800-3400K) and as such it is unsuitable for daylight film with its color temperature of 5500-6000K. In order to achieve the proper color rendition, a light balancing filter is used, and the tungsten light is converted to daylight.

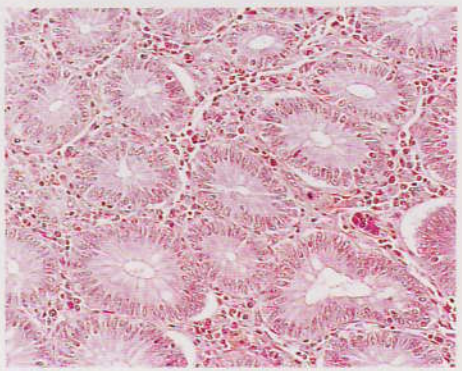
Differences in color reproduction depending on differences in color temperature



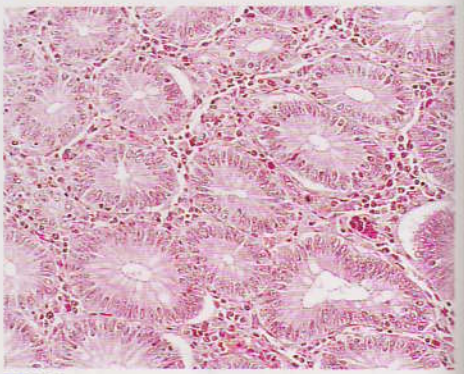
4000K



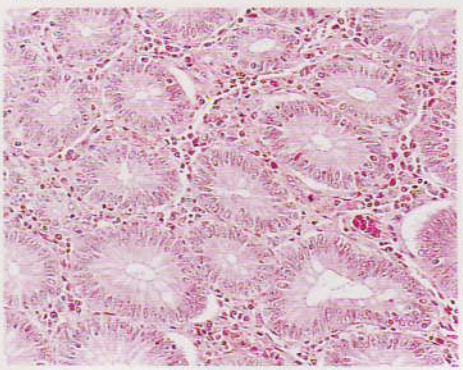
4500K



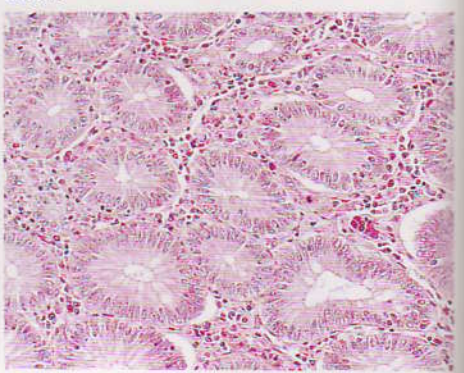
5000K



5500K

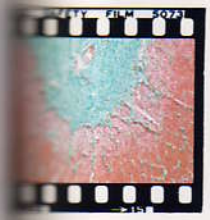


6000K



6500K

(If color temperature is low, the photo shows a "red shift", and if color temperature is high, it exhibits a "blue sh



Differences in color rendition depending on the type of film

Color rendition for different types of film from the same manufacturer tends to vary, and even different production lots of the same type show slight differences.

When you start to take color photos, you should first determine the optimum conditions for the film through test photographs, matching them with both microscope and specimen.

(For the method of taking test photos refer to page 54)

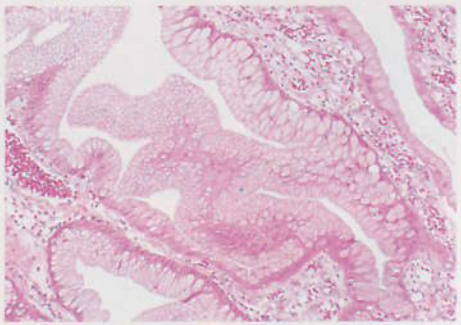
Differences in color development depending on the type of film

The six photos on the right were taken with films of different brands, and with the exception of the ISO/ASA speed all photographic conditions were identical. This example clearly shows that properties such as color rendition, contrast, clarity of background, etc. are all different because of the various brands of film.

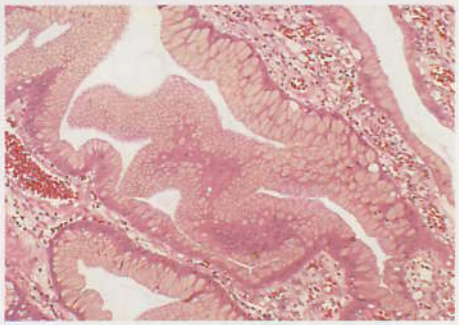
Photographic conditions

BHS, PM-10AD, LBD-2N (color temperature 5500K), LBT (color temperature 3400K), shutter speed 0.1-0.4 sec

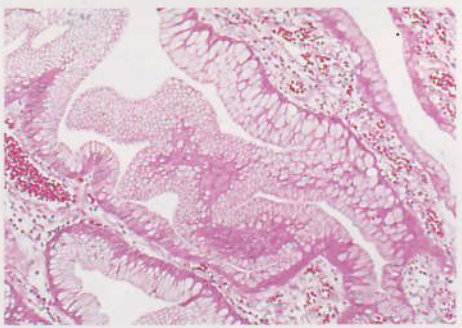
Daylight type



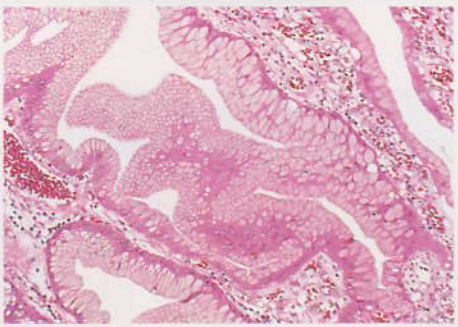
A



B

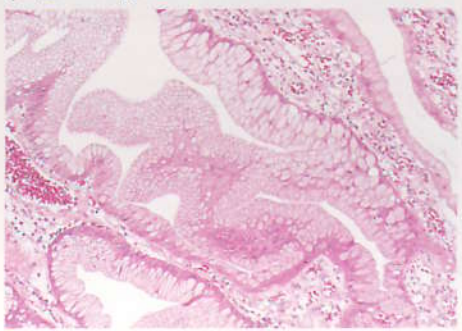


C

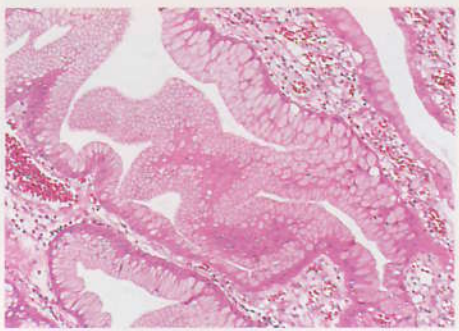


D

Tungsten type



E

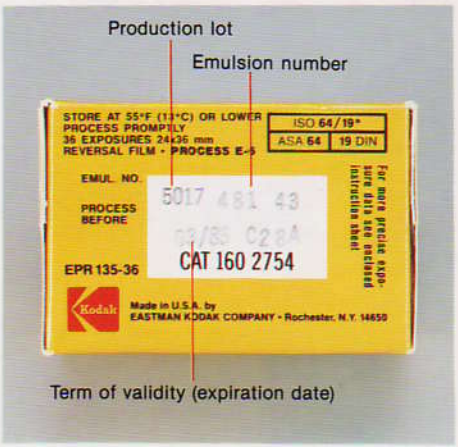


F

Purchase of color film

1. What to watch for when buying color film

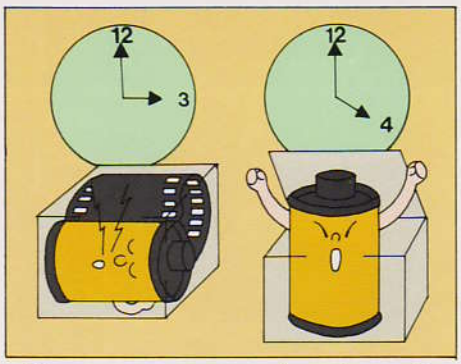
- Color film is a highly sensitive substance, and environmental factors such as heat and humidity easily cause changes in film speed and color rendition. When buying color film, avoid camera stores in which the shelves storing color film are exposed to sunlight.
- Choose only color film with a sufficiently long period before the expiration date as marked on the package.
- Color rendition of the same film type may differ, if it comprises different production lots. If you frequently use color film, you can use it under identical photographic conditions and avoid variations in the color of the film if you buy large quantities from the same production lot.



2. How to store film

For the basic principles of composition and color rendition check the technical literature on the subject. As has been pointed out, performance changes according to the conditions under which the film is stored.

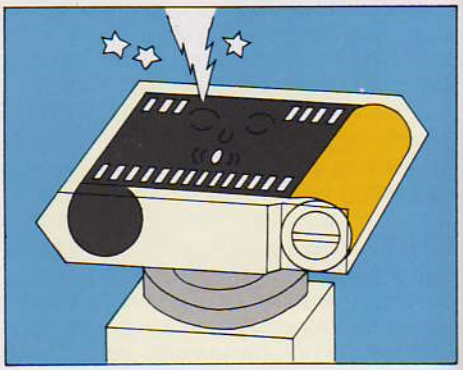
Color film is normally kept in the refrigerator to protect it from the effects of heat and humidity. Remove the film from the refrigerator 1 hour before use and allow it to reach room temperature. Do not remove the film from the roll immediately after taking it out of the



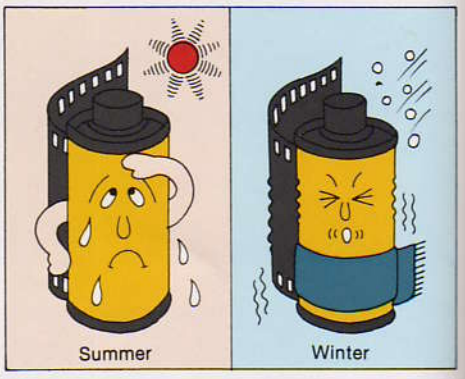
refrigerator to prevent condensation on the film surface.

3. Points calling for special attention when using the film

- Do not leave the film in the camera longer than necessary.



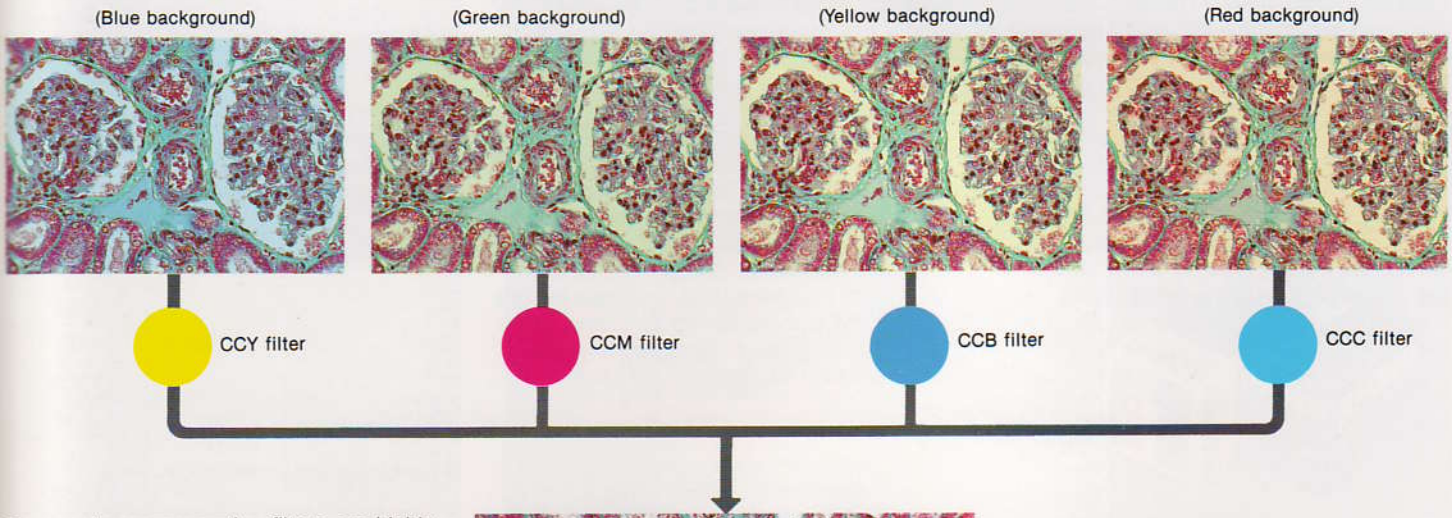
- Develop exposed film as soon as possible.



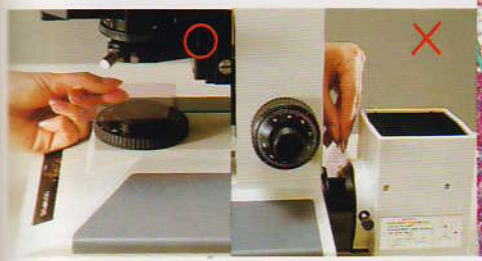
- Even though other photographic conditions may be identical, color rendition may vary with low or high ambient temperatures.
- Do not use the film in a gaseous environment (such as formalin), since color rendition will be adversely affected.

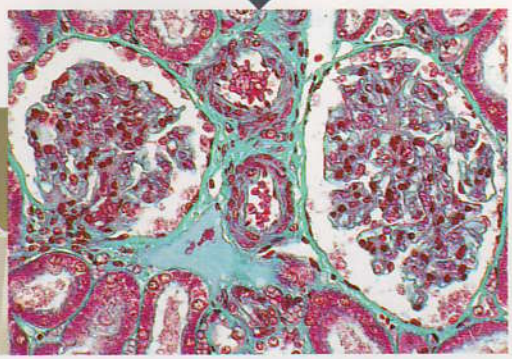
Use of color-compensating (CC) filters when taking color photos

Color-compensating filters used



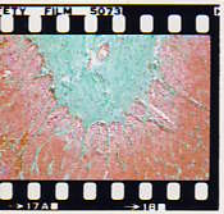
Since color-compensating filters are highly sensitive to heat, do not use them close to the light source.





- CC filter is the abbreviation for color-compensating filter, and the color of the particular filter is marked in capital letters on its rim. Six different colors are available, each color being offered in six different degrees of density. No. 5 and No. 10 are most widely used in photomicrography.
- Color filters are made by several manufacturers. Check your Olympus dealer or a reputable camera store for availability.

Color to be reduced	Color-compensating filter required	
BLUE	Yellow	CCY
CYAN	Red	CCR
GREEN	Magenta	CCM
YELLOW	Blue	CCB
RED	Cyan	CCC
MAGENTA	Green	CCG



Test photography

1. The need for test photography

Even if you use automatic photographic equipment and set both the ISO/ASA speed and the reciprocity law failure characteristics, you cannot be sure that you will always achieve the perfect exposure for every type of specimen. The reason for this is that photographic equipment is manufactured to match distribution state, color, and exposure standard of average specimens. In order to obtain better results, you should first take test photographs to match the conditions of the specimen, film, microscope, filters, etc.

2. Method for test photography

(when using models BHS and PM-10AD and reversal film)

(1) Prepare one roll of film (36 exposures) and a frequently used specimen.

(2) Set the test conditions (see chart 1) and prepare a data chart (see chart 2). Listing all the conditions given in chart 1 will result in a data chart 2. Take your photos based on this chart and determine the optimum photographic conditions on the basis of the results obtained on the developed film.

**For color compensation, first take photos without a compensating filter and then choose the proper compensating filter on the basis of the finished photo.*

Chart 2: Data chart

	Color temperature	Exposure	Color compensation
1	5900K or 10V	No compensation	0.8X
2			1X
3			1.25X
4		CC5G	0.8X
5			1X
6			1.25X
7		CC5M	0.8X
8			1X
9			1.25X
10	5500K or 9V	No compensation	0.8X
11			1X
12			1.25X

Chart 1: Test conditions

Color temperature	Measuring with CTR	170 mired, 5900K / 180 mired, 5500K / 190 mired, 5260K
	No CTR	Voltage 10V, 9V, 8V
Exposure	Exposure adjustment	0.8X, 1X, 1.25X
Color compensation	Color-compensating filter	CC5G, no compensation, CC5M

3. Evaluating the test photos

Observation of reversal film transparencies can be carried out either with a light box or a slide projector, but the display of color varies widely with the color temperature of these light sources. As a standard for evaluating the proper color, use a light box with a color temperature close to 5000K.

The following types of light boxes are available on the market:

- ○ Fuji film color box 5000
- ○ Macbeth Prooflite
- ○ Durotest Color Classer 50
- ○ General Electric Chroma 50

4. Organizing photographing data

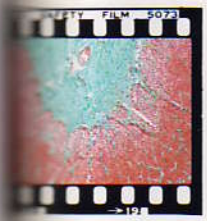
Make it a habit to record all data relating to both test photography and regular photography. If the photographic conditions that resulted in good photos are retained on file, troubleshooting and correction of problems can be carried out quickly and efficiently.

(Pages 40-41 give an example of a data report form for your reference)

5. Storing developed film

When storing film for a long period of time, the most important precaution is protection from light. Since mold is prevalent in hot and humid places, it is advisable to keep the film tightly sealed, together with a desiccant, such as silica gel.

We recommend storing film in an environment with a relative humidity of 15-40% and an ambient temperature below 21°C (71°F). (from Kodak Color OKP 141 instructions).



Black-and-white photography

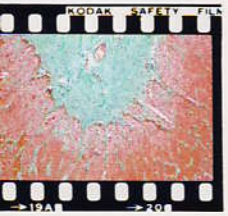
Black-and-white film

Since the conditions differ for photomicrography and general photography, it is necessary to select the proper film for each type of photography. In photomicrography, high contrast film with fine grain is used in order to document minute structures of biological specimens and to achieve sharp photographic reproduction. Examples of this film type are Kodak Panatomic X, Agfapan 25, Ilford Pan F, and Fuji Neopan F.

Use of black-and-white film

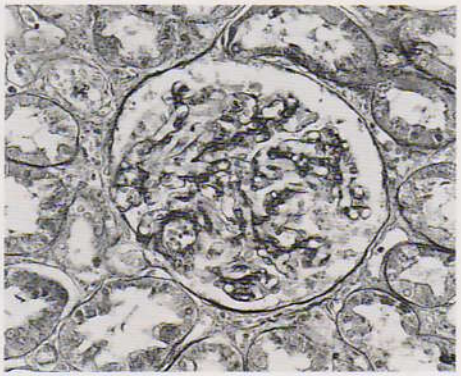
There are many types of black-and-white film, and the key to obtaining good photographs lies in selecting the right film for a particular job.

Specimen conditions	Film brand	ISO/ASA	Remarks
Normal or high contrast for the stained section of general pathological specimens	Kodak Panatomic-X Ilford Pan F Agfapan 25 Fuji Neopan F	32 50 25 32	The use of contrast filters depends on the color of the specimen.
Low contrast for the stained sections of pathological specimens, as well as those of other areas. When shape is more important than structure details.	Kodak Technical Pan 2415 Agfaortho 25 Fuji Minicopy HR-II	64 100 25 32	Since the contrast of the film is very high, the range of proper exposure is very narrow. Unless exposure is set within ±1/3 step of the optimum, the photo will be either over- or underexposed.
Dark specimens, and when long shutter speed is required	Kodak Tri-X Pan Agfapan 400 Ilford HP5 Ilford XP1 Fuji Neopan 400	400 400 400 50-1600 400	Use these types when you want to shorten exposure time at the expense of film grain.

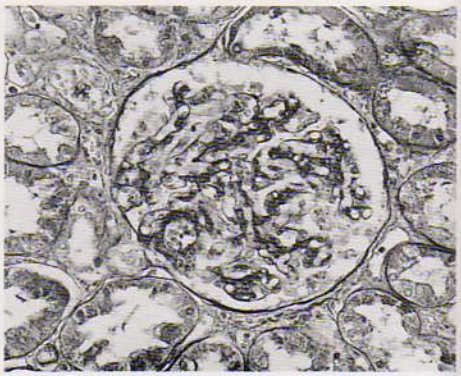


Comparison of different film brands

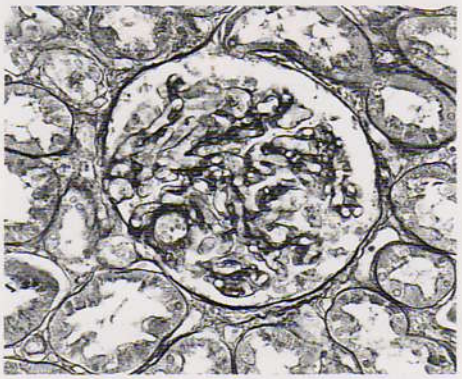
Types of film providing a wide gradation from shadow to highlights and featuring good contrast are Fuji Neopan F and Kodak Panatomic X. Technical Pan offers slightly higher contrast, while Mini Copy eliminates grey tone details.
Neopan SS and Tri X, on the other hand, have fairly low contrast.



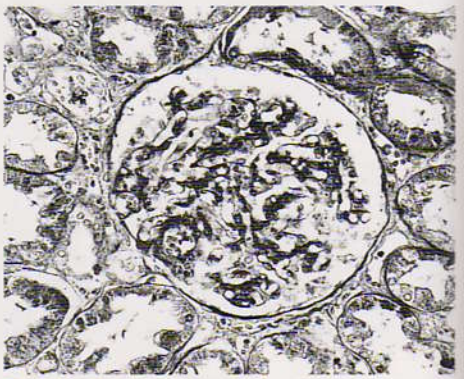
Fuji Neopan F



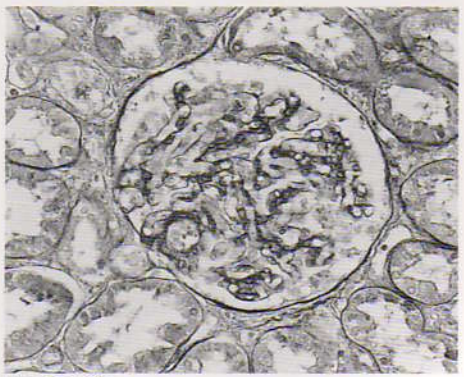
Kodak Panatomic-X



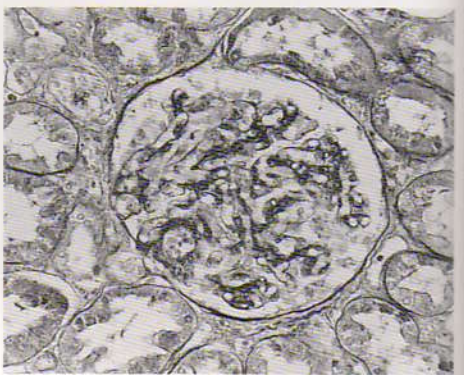
Kodak Technical Pan 2415



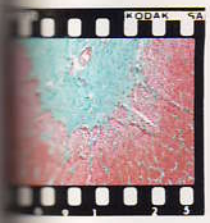
Fuji Mini Copy



Neopan SS



Kodak Tri-X



Filters

Use of contrast filters

Contrast filters are used to control the contrast of black-and-white photos.
For black-and-white photomicrography, green filters are normally used, but choice of the most effective filter depends on the type of specimen. Filters that enhance the colors and contrast of the specimen are:

Color of the specimen	Color of the filter
Red/yellow	Green
Yellow/orange	Blue
Blue	Orange

For reducing contrast, use a filter of the same color as the specimen.

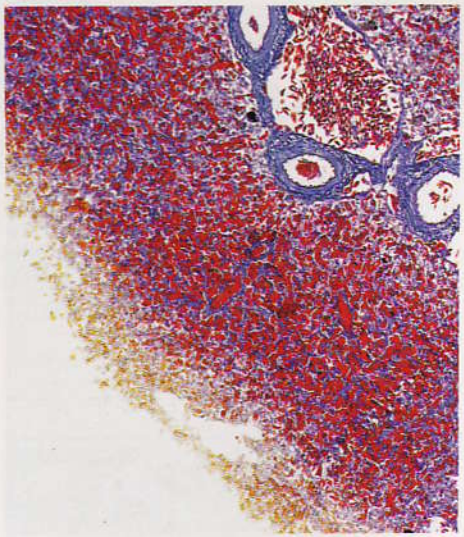
Reasons for using a green filter

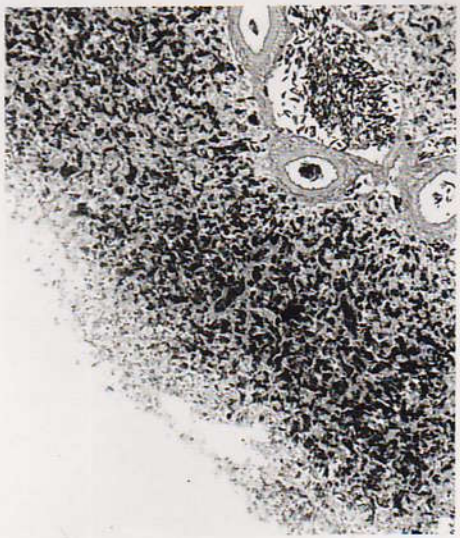
There are two reasons why contrast improves when a green filter is used.

(1) Since objective aberrations are most effectively compensated near the green wavelength, loss of image clarity due to chromatic aberration is averted by a green filter.

(2) Dyes such as hematoxylin and eosin absorb green light well, resulting in higher contrast when a green filter is used.

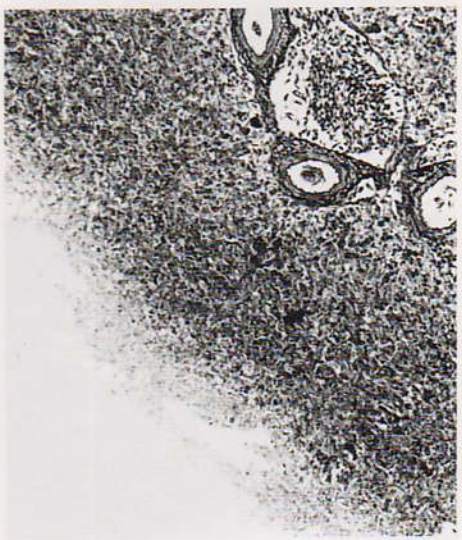
Effects of various contrast filters



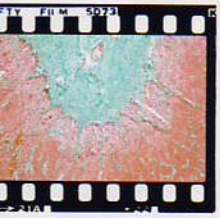


Without filter

Green filter:
the red portion is enhanced



Orange filter:
the red portion is reduced



Photography with Polaroid® film

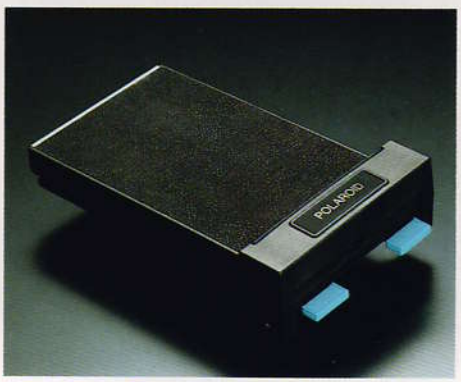
One of the advantages of Polaroid film is that it can be quickly viewed as a finished photo, but it is seldom possible to obtain color reproduction comparable to that of 35mm reversal color film.

1. Types of film used in photomicrography

	4 1/4″ × 3 1/4″ (actual size 7.3cm × 9.5cm)			4″ × 5″ (actual size 9cm × 11.5cm)			
	Type	ISO/ASA speed	Pack containing 8 photos	Type	ISO/ASA speed	Sheet	Pack containing 8 photos
Black-and-white	107	3000	○	52	400	○	—
	667	3000	○	57	3000	○	—
	665 (Print/ negative)	50	○	552	400	—	○
				55 (Print/ negative)	50	○	—
Color	108	80	○	58	80	○	—
	668	80	○	59	80	○	—
	669	80	○	559	80	—	○

■ 4″ × 5″ film holders differ according to the type of film

Sheet film—545 type film holder



Packed film—550 type film holder

2. Photographic techniques

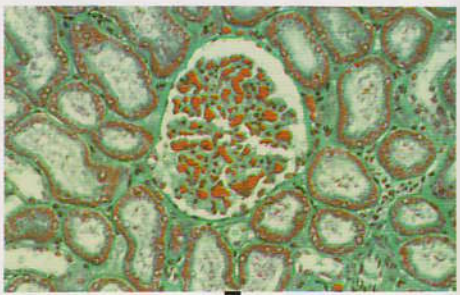
(1) *Black-and-white photography*

Use a green filter to obtain good contrast.

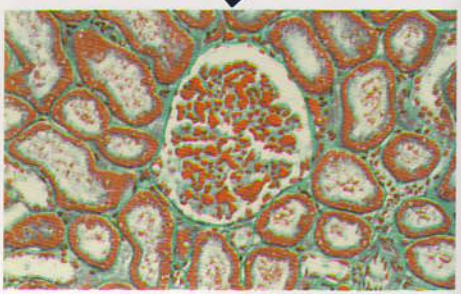
(2) *Color photography*

Since the overall color hue tends to be either light green or blue, use CC10 ~ 20M or CC10 ~ 20Y for compensation. If you want to enhance color contrast, use the FF filter available from your Olympus dealer.

Since Polaroid film is more easily affected by reciprocity law failure than normal film, exposure time should be adjusted to between 0.05 and 0.5 seconds.



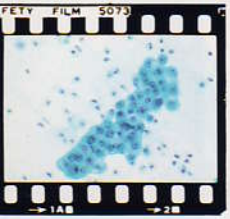
Color temperature 4500K—LBD-2N



Color temperature 4500K—LBD-2N plus FF filter

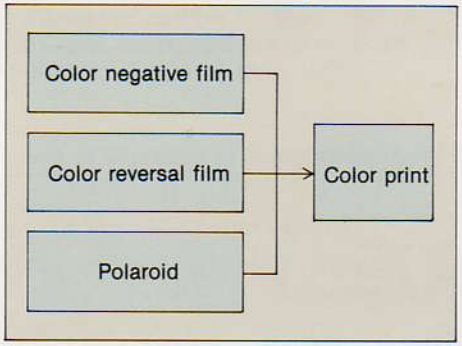
Section 3

How to obtain good prints



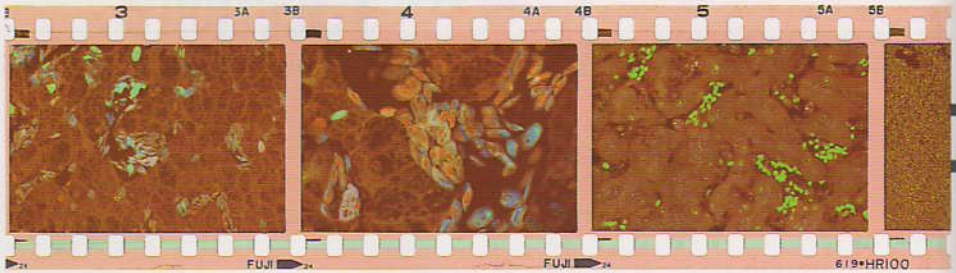
How to obtain good color prints

The following diagram shows how to get good color prints (positive prints).

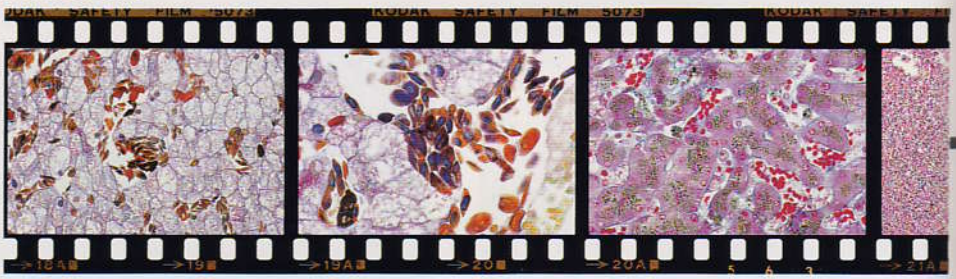


In photomicrography, the color reproduction of finished prints taken with negative film differs from the observed color—a fact many users are probably very much aware of. Possible reasons for this are that the technician who made the prints did not view the specimen color through a microscope and thus did not know the actual color, or the photographer made a mistake when he used a filter and failed to notice that the film quality deteriorated. In order to get better results, you should first photograph the specimen from which you want to get prints on reversal film, and attach the finished photo as color reference sample to the negative film. Prints can also be made from color reversal film, but the process does not match the print quality as obtained from negative film.

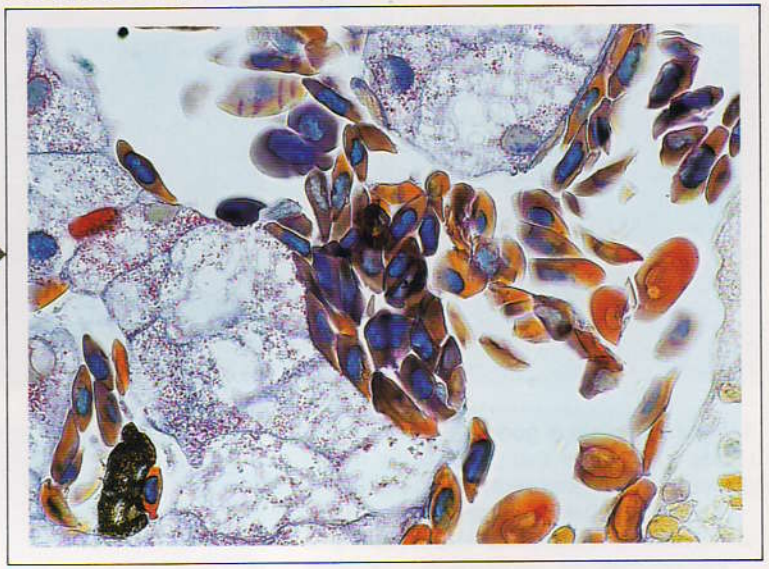
Color negative film



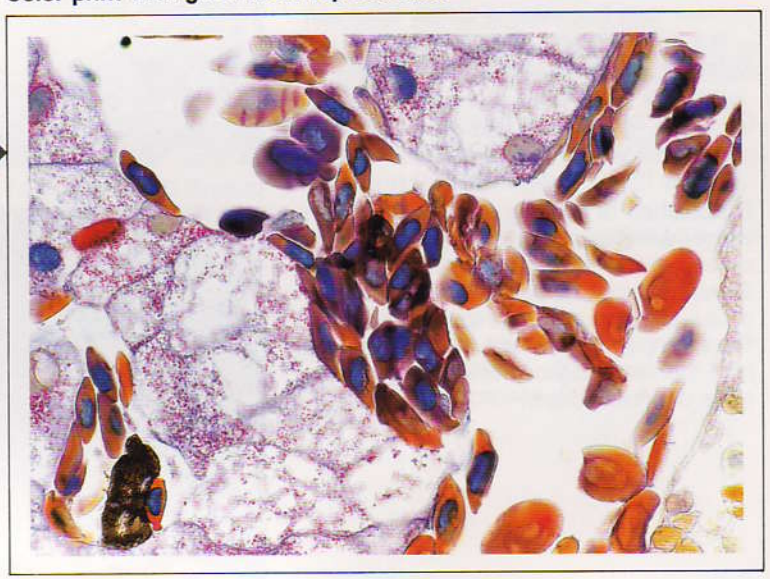
Reversal film



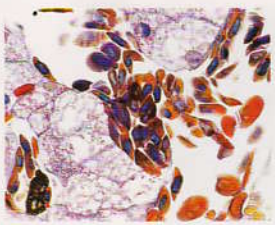
Color print with poor color reproduction

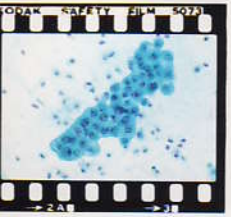


Color print with good color reproduction



Reversal film with optimum color rendition





Preparing black-and-white prints

There are many technical publications on the market that explain in detail how to develop film. If you plan to do your own film processing, use these books as reference. But since contrast in photomicrography is lower than with normal photographic subjects, you should pay particular attention to underdevelopment. If you make a positive print from an underdeveloped, low-density negative, an even greater reduction in contrast will occur, leading to a poor result.

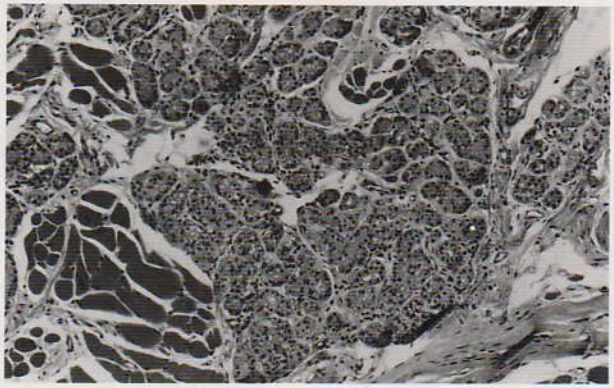
1. How to obtain a good negative

(1) Use a developer that matches the film
(2) Use the developer at the temperature specified by the manufacturer
(3) Adhere to the specified development time that matches film sensitivity
(4) Agitate gently and frequently to eliminate uneven development
(5) Make sure that there is neither too much nor too little fixing
(6) Follow the paper specifications when washing the print
(7) Handle the negative carefully and protect it from fingerprints, scratches and dust

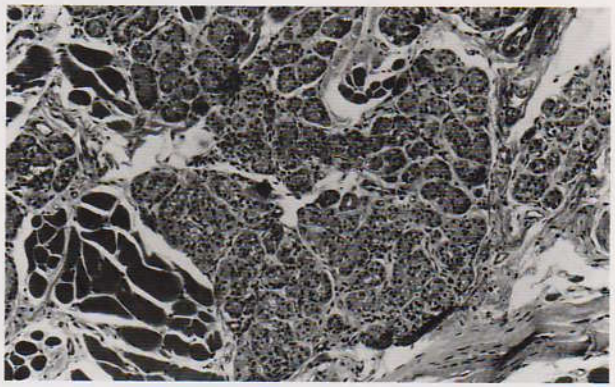
2. Selection of photographic paper

When making prints, you can vary the contrast depending on the type of printing paper. Select the type that best suits both the density of the negative and specimen contrast. The examples on the right show prints made from the same negative when using different types of printing paper.

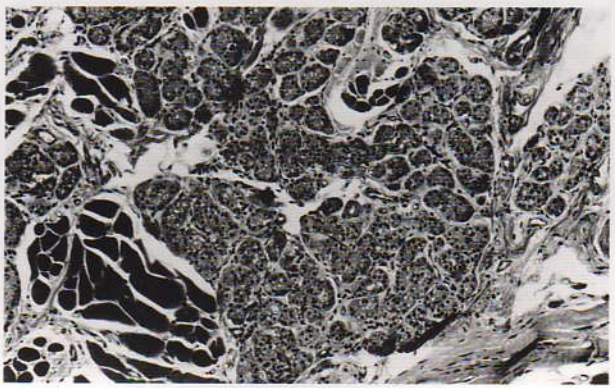
Grade no. of photographic paper



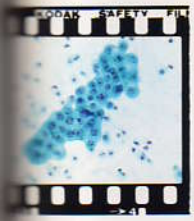
Grade 2—low contrast



Grade 3—normal contrast



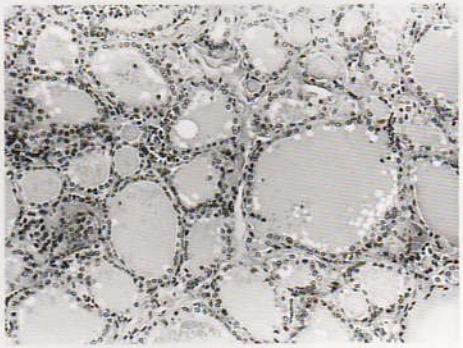
Grade 4—high contrast



Marks on the photo

Marks on the print tend to stand out more prominently in photomicrography than on normal photos of people and landscapes. Since photomicrography employs film with fairly high contrast, even the most minute differences in brightness show up in the picture. Marks resulting from improper development are also very conspicuous. This section deals with marks resulting from development.

1. Marks as a result of negative development

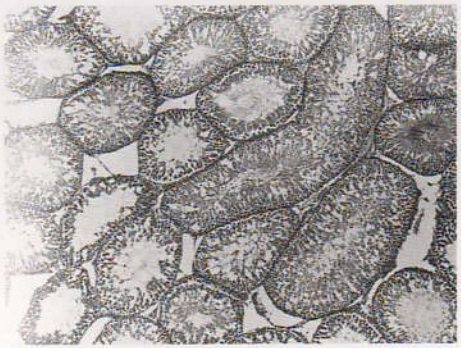


Both upper and lower portions of the photo are brighter than the central section.

How to avoid creating marks

(1) Confirm the degree of exhaustion of the developer.
Carefully note production date and frequency of use of the developer.
(2) Agitate the developer thoroughly before use.
(3) Use the developer at the specified temperature.
(4) Do not work with an exposure that results in a development time of less than 5 minutes (the result will be extremely desensitized development).
(5) Agitate the bath thoroughly during development.
(6) Maintain the specified fixing time.
(7) Wash the film thoroughly.

2. Marks occurring during print development

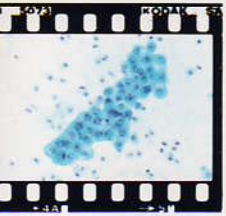


Marks with irregular brightness show up.

If you use an automatic processor to develop the printing paper no marks will occur, but since marks can easily show up when developing in a tray, the following points should be noted:

How to avoid creating marks

(1) Agitate the developer thoroughly.
(2) Keep the temperature of the developer at 20°C, or as specified by the manufacturer.
(3) Since marks can easily occur if development time is too short, set the exposure of the enlarger at such a level that development time is between 1 min 30 sec and 2 min.
(4) Agitate the bath thoroughly during development.
(5) Continue to agitate thoroughly even while stopping and fixing.
(6) Wash the print thoroughly in running water.
Regular-base paper—30-60 min
Resin-coated paper—4-5 min

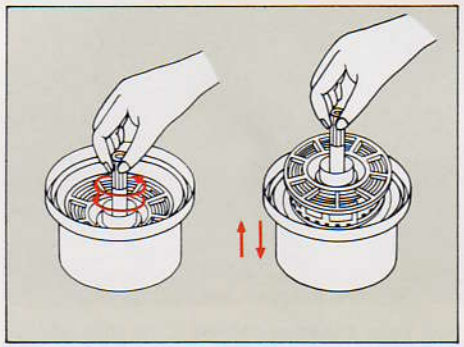


How to avoid marks during development

Development of film

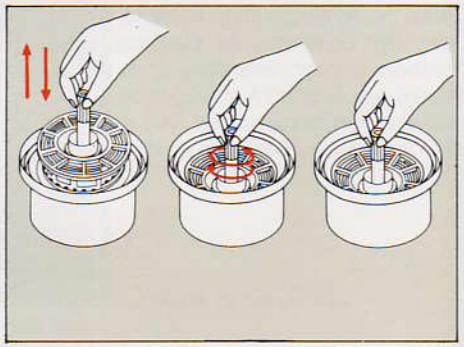
Since marks on the film occur easily when using a reel-type tank, development should be done in the following manner in the dark-room.

1



Immerse the reel in the developer, and after turning it 2-3 times, tap it another 2-3 times against the bottom of the tank to remove air bubbles sticking to the film surface. Complete this process within 5-6 seconds.

2

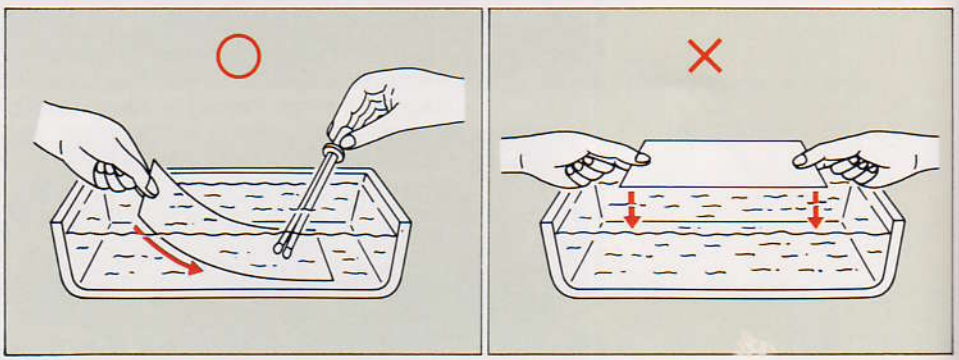


Now remove the reel from the developer and immediately immerse it again. After repeating this procedure, turn the reel immersed in the developer 2-3 times, then pause for 30 seconds.

Development of printing paper

This section deals with development using a tray.

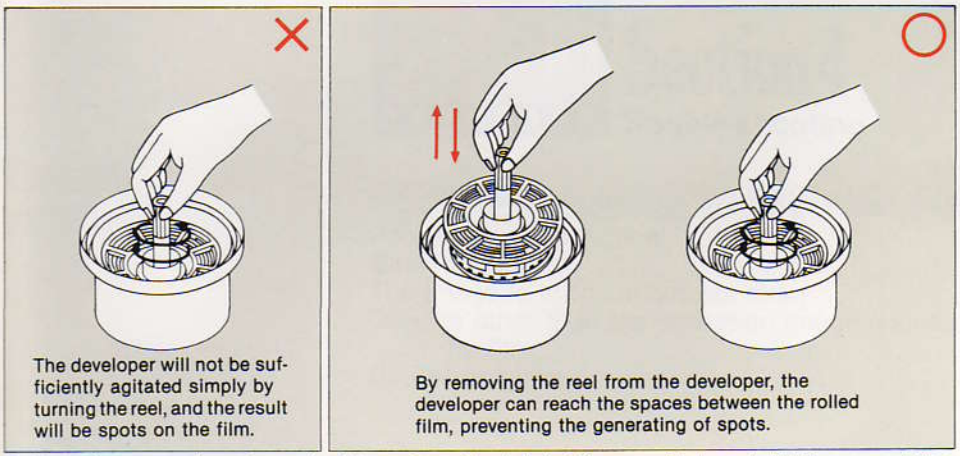
1



While holding the edge of the exposed printing paper lightly with a pair of tongs, quickly place it into the developer.

■ As shown in the picture, do not immerse the printing paper into the developer parallel to the developer surface, but tilt it into the developer bath.

3



The developer will not be sufficiently agitated simply by turning the reel, and the result will be spots on the film.

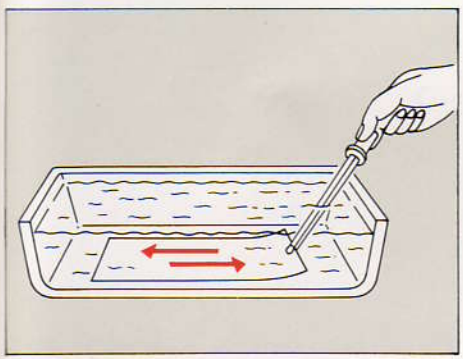
By removing the reel from the developer, the developer can reach the spaces between the rolled film, preventing the generating of spots.

Perform the operation shown in picture 2 on the left in 30-second intervals till the completion of development work.

4

After completing development, follow the sequence of stopping, fixing, and washing at the proper time.

2



To agitate the photographic paper, hold it at a corner with a pair of tongs and move it back and forth while it is submerged in the developer. Repeat this operation in 15-second intervals until development work is completed.

- Make sure that the photographic paper does not float up to the surface of the developer.

3

After completing development work on the printing paper, quickly perform the sequence of stopping, fixing, and washing.

- Make sure that separate sheets of printing paper do not stick together in the fixer.
- Do not use the same pair of tongs during stopping and fixing that you used during development work.
  Make sure not to contaminate the developer with either stop bath or fixer liquid.

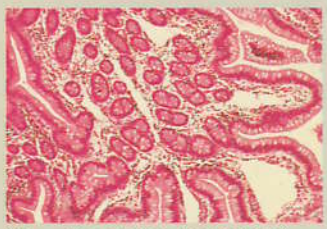
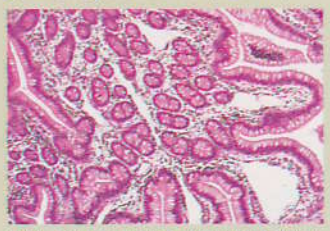
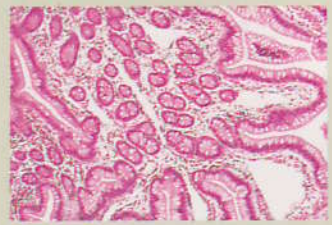
Section 4
Trouble-shooting

Problems in finished photos and their correction

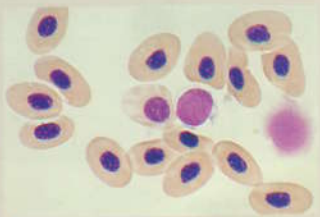
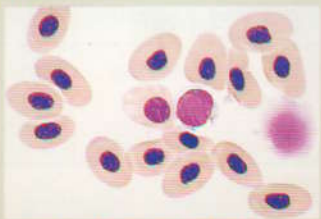
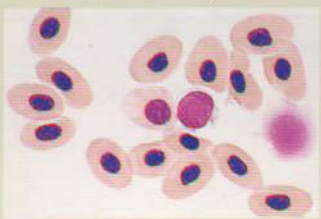
Problems in finished photos and their correction

—Poor color reproduction

1. The background is colored (red/blue)

Cause	Correction	Remarks	Refer to page
The color temperature of the illumination is not matched to that of the film. ●Light balancing filter specified by manufacturer is not used.	●Use filters specified by the manufacturer.	●Daylight type: LBD-2N, 5500K, D setting ●Tungsten type: LBT, 3400K, T setting	Page 48/ page 50
Lamp voltage is too low (too high).  6V—voltage is too low (red hue).  11V—voltage is too high (blue hue).	●Raise (lower) lamp voltage  9V—correct value	●BHS above 8.5V ●BHT and BHTU at 5~6V ●If you have reached the desired color temperature, do not change the voltage position. If you want to change intensity, use an ND filter. ●We recommend that for test photography the voltage should be set to the standard position ±1V when first determining the optimum color temperature.	Page 50/ page 54
Blackening of lamp due to prolonged use	●Replace with a new lamp		

2. The background is colored (green/magenta)

Cause	Correction	Remarks	Refer to page
●A film different from your usual film type was used.	●Since color rendition varies with different film types even from the same maker, choose a film with coloring suitable for your type of work.		Page 51
●A film of the same type but with different emulsion number was used.	●If possible buy film with the same emulsion number in large quantities.	●In order to maintain the performance quality of the film, store it in a refrigerator and remove it one hour prior to use, allowing it to reach room temperature. ●Even for film of the same type there will be slight variations in color reproduction depending on laboratory, development conditions, and staining of the specimen.	Page 52
 Greenish background	●Use a color-compensating (CC) filter.  CC05M—optimum compensation  CC10M—over-compensated	*Color-compensating (CC) filters Buy from your photo dealer. ●Too much green Use CCM (magenta) ●Too much pink Use CCG (green) For photomicrography, use CC filters 05-10. For further compensation use CC05 and CC10 together.	Page 53

3. Incorrect color rendition

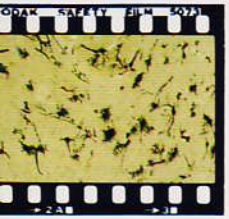
Cause	Correction	Remarks	Refer to page
●An excessively long exposure time has been used and the characteristics of reciprocity law failure lead to incorrect color rendition.	●Set shutter speed at 0.01-0.05 sec. Adjust shutter speed uniformly as far as possible by using an ND filter. ●When using long exposure times (above 0.5 sec) with models PM-10AD and PM-10ADS, set the characteristics number of the film used on the dial for reciprocity law failure.	●With long exposure times, even with the exposure time compensated, the color rendition changes as a result of film properties. Use a color-compensating filter specified by the film manufacturer.	Page 46
●Automatic exposure has been used without adjustment.	●When using models PM-10ADS/ PM-10AD set the exposure adjustment dial to: 0.8-0.25X for bright background specimens and to 1.25-4X for dark background specimens. ●Carry out exposure adjustments Bright background: ISO/ASA 100 to 50 Dark background: ISO/ASA 100 to 200	●If you change the magnification of the objective, the light distribution within the visual field will also change.	Page 42/ page 43
●Actual and nominal sensitivity of the film differ.	●Vary the ISO/ASA speed setting.	●Nominal speed may differ from actual light sensitivity as much as 1/3 to 1/2 aperture.	Page 42

4. Poor color of a print enlarged from a negative

Cause	Correction	Remarks	Refer to page
●The printing technician in the laboratory is not sure of the subject's correct color.	●Add a transparency of the same specimen with correct color as a sample.	●Better color is achieved when shooting a transparency with reversal color film (but the cost of prints goes up and quality decreases).	Pages 60-61

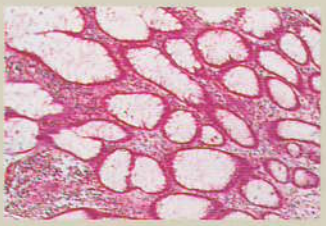
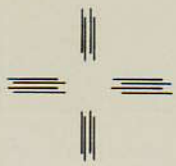
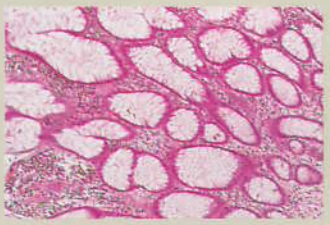
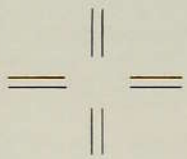
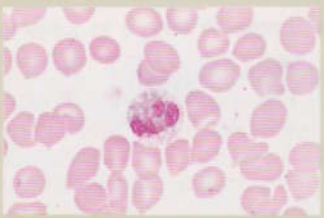
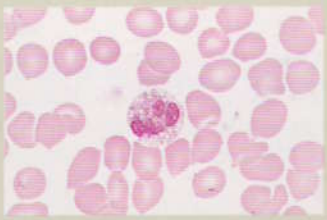
5. Poor color of Polaroid film

Cause	Correction	Remarks	Refer to page
●Wrong color temperature.	●The film is a daylight type, but because of the film characteristics color temperature should be set lower than for 35mm film.	●If you set color temperature between 4000K and 5000K and use an Olympus FF filter, color contrast will be enhanced.	Page 58
●Abnormal color characteristics.	●If the abnormalities are within the adjustment range for color temperature and within the operational range of the color-compensating filter, determine the conditions by test photography.	●Since Polaroid film is easily affected by adverse storage conditions as far as color characteristics are concerned, protect it from heat and humidity by storing it in a refrigerator.	Page 54/ page 58
●Term of validity of the film has expired.	●Use film whose term of validity has not yet expired.		
●If room temperature is abruptly lowered (raised), sensitivity is affected and print color turns towards blue (red).	●For development time and color reproduction, refer to the film instructions.		



Blurred image

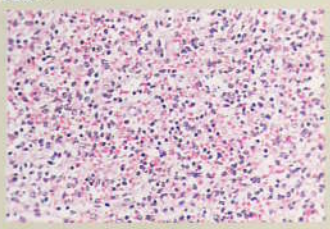
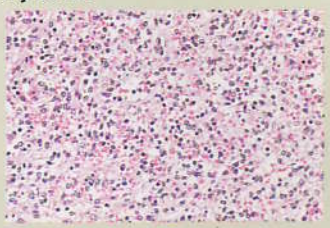
1. The overall focus of the picture is blurred

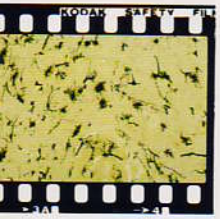
Cause	Correction	Remarks	Refer to page
●Finder eyepiece or focusing telescope is not properly adjusted.  	●Adjust the diopter until the double cross lines are clearly visible.  	●Since most people do not have the same visual acuity in both eyes, determine which eye you always use for focusing.	Pages 26-27
●Blur and drift resulting from vibrations.  Image affected by vibrations	●Use a vibration-proof table.  No vibrations ●Use an ND filter and increase shutter speed (1/2 sec to 1 sec). ●Use a stand for the photographic equipment to separate photographic equipment and microscope.		Page 12 page 48

2. Focusing error occurs when you use a low-magnication objective of less than 4X

Cause	Correction	Remarks	Refer to page
●If magnification is low, focal depth at the film plane becomes shallow, easily causing errors in focusing.	●Mount a focusing magnifier to the finder eyepiece or the focusing telescope, and after focusing on the double cross lines, fine focus the specimen.	●Increasing the magnification eliminates focusing errors.	Page 27

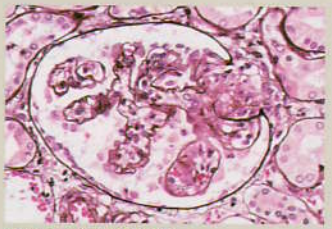
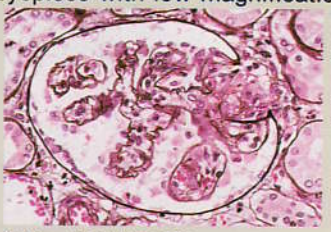
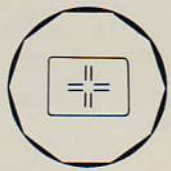
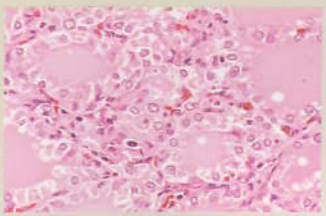
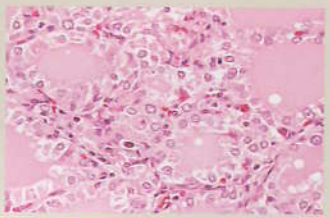
3. The periphery is uniformly blurred

Cause	Correction	Remarks	Refer to page
●An Achromat type objective was used.  D Ach 10X NFK 2.5X	●Use a Plan Achromat type objective  D Plan 10X NFK 2.5X		Page 14
●Objective and photo eyepiece were used in the wrong combination.	●LB series (long barrel)—NFK photo eyepiece ●Short-barrel series—FK photo eyepiece		Page 13



The image is in focus but not sharp

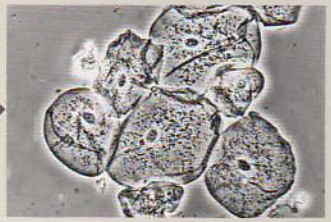
1. Inadequate resolving power

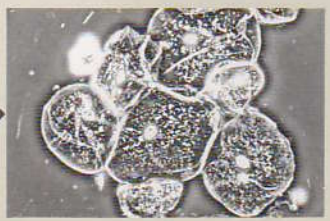
Cause	Correction	Remarks	Refer to page
●Use of a combination of low-magnification objective and high-magnification photo eyepiece.  S Plan 20X NFK 5X	●In order to obtain high resolution, use an objective with large numerical aperture and a photo eyepiece with low magnification.  S Plan 40X NKF 2.5X	●In order to obtain a magnification of 100X on the film plane, use either an objective 40X with a photo eyepiece of 2.5X or an objective 20X with a photo eyepiece 5X. To increase resolving power, the combination of objective 40X and photo eyepiece 2.5X is preferable. (Focal depth, however, will become shallow.)	Page 38
●Use of the condenser with the aperture iris diaphragm fully opened.	 ●Stop down the aperture iris diaphragm to 60-80% of the numerical aperture of the objective.	●Vary the amount by which you reduce the aperture iris diaphragm according to the magnification of the objective and the contrast of the specimen.	Pages 24-25
●The field iris diaphragm was fully opened.	 ●In order to reduce stray light, stop down the field iris diaphram to an area only slightly larger than the frame reticle.	●Do not reduce the diameter of the field iris diaphragm to such an extent that it touches the frame reticle because the actual area photographed is always slightly larger than the area within the frame reticle.	Page 23
●Use of thick cover glass.  Cover glass 0.2mm	●Use a cover glass with a thickness of 0.17mm.  Cover glass 0.17mm	●Olympus objectives for biological specimens have been designed in such a way that optimum resolving power is obtained when a cover glass with a thickness of 0.17mm is used.	

Cause	Correction	Refer to page
●The specimen stain is too weak, resulting in lack of contrast. (Photographing a specimen that cannot be stained)	●Use a denser stain. ●Use a contrast filter (for black-and-white photography only). ●If the specimen cannot be stained, use phase contrast, differential interference contrast or darkfield to create contrast optically.	Page 57

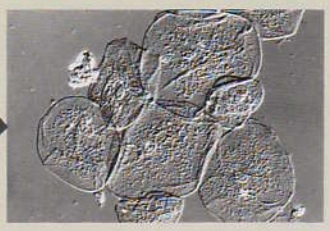
Brightfield

Phase contrast

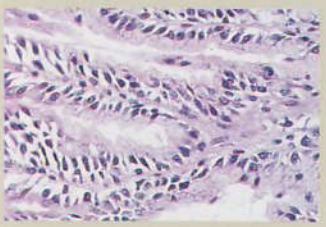
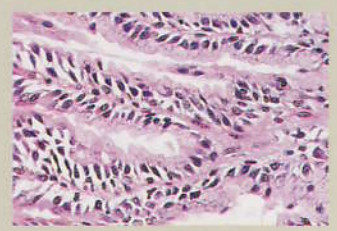




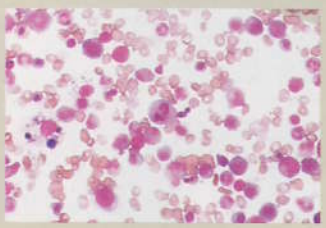
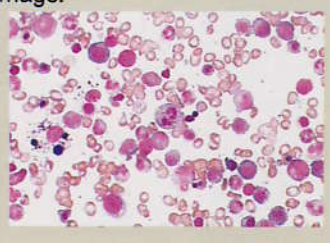
Differential interference contrast

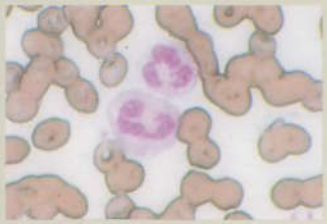
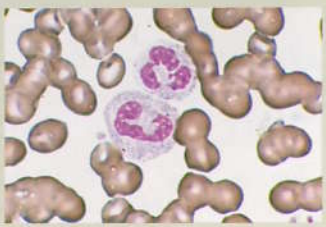
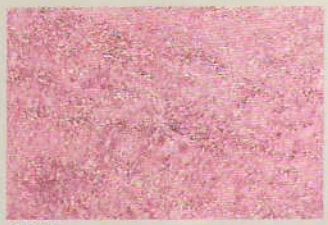
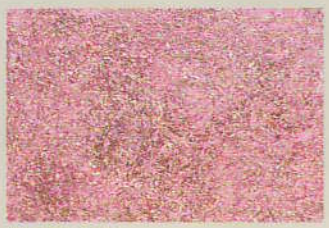
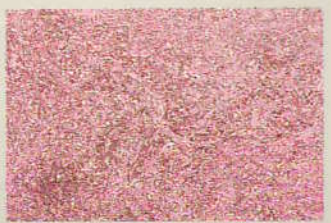


Darkfield

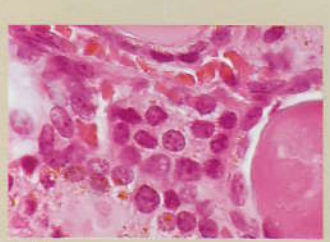
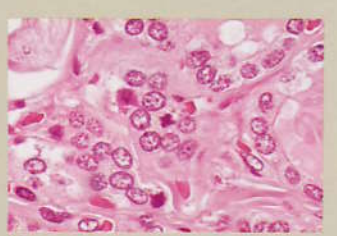
Cause	Correction	Remarks	Refer to page
Color photography ●Use of a low contrast film.  Ektachrome 200	●Use a high contrast film.  Kodachrome 25		Page 51
Black-and-white photography ●Variations in the spectral sensitivity of the film affect the contrast.	●Normally, a green filter is used, but if you want to emphasize a specific portion of the specimen, use another contrast filter.	●If a filter complementing the color of a specimen is used, it will emphasize the contrast.	Page 57

2. The image appears hazy

Cause	Correction	Remarks	Refer to page
●The correction collar of the objective is not adjusted to the thickness of the cover glass. 	●Adjust the correction collar while examining the specimen and set it at a position providing a clear image. 	●On the LB objective a correction collar is mounted on the S Plan Apo 40X, S Plan 100X dry and D Plan Apo 60X.	Page 16

Cause	Correction	Remarks	Refer to page
●An objective normally used with cover-glassed specimens was used on a specimen without cover glass (or vice versa). 	●Use a no-cover objective. 	LB objectives ●NC S Plan 40X ●NC D Plan FL 60X ●NC S Plan Apo 100X oil ●NC S Plan 100X dry	Page 17
●Fingerprints, an oil film or dirt particles in the optical system (objective front lens, photo eyepiece, prism, specimen, etc.)  Oil film  Fingerprints	●Clean the optical system. ●Always cover the microscope with a dust cover when not in use.  Clean the optical system.	●If you use dry 40X or 60X objectives together with an oil-immersion objective, the oil on the specimen may soil the front lenses of the dry objectives.	Pages 26~32

3. No sharp image is obtained with a 100X oil-immersion objective

Cause	Correction	Remarks	Refer to page
●No immersion oil was used.	●Use oil specified by the manufacturer.		Page 18
●Unsuitable oil was used.	●Use specified oil, since the types used for fluorescence and normal white light often differ.		Page 18
●The oil contained air bubbles.	●Apply the oil after you have removed bubbles in the bottle.	●Remove the eyepiece before examination and look through the eyepiece sleeve.	Page 18
●Using oil in a room with unsuitable temperatures (too high or low)	●If room temperature is either too high or too low, or if the air is too humid, the diffraction index changes, causing changes in the image. Use the oil at a room temperature of 22-25°C and at a humidity of about 55%.		Page 12
●The specimen is too thick.  4 ~ 5μ	●It is advisable to use a specimen with a thickness of 2-3μ.  2μ		

4. Entire roll of black-and-white film is not sharp

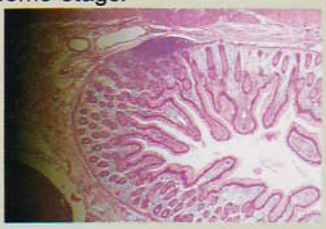
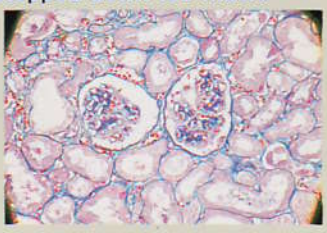
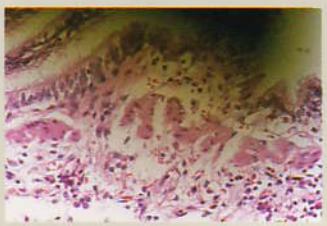
Cause	Correction	Remarks	Refer to page
●Possible causes are: type of film, emulsion, overexposure, over-development, improper handling or accidents during development, etc.	●Check these possibilities and correct them.		Pages 55 ~ 57 Pages 64 ~ 65

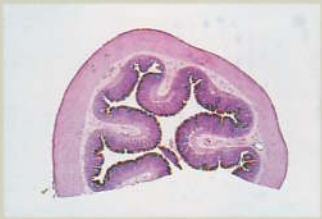
5. The finished print appears grainy

Cause	Correction	Remarks	Refer to page
●A film with coarse grain was used.	●Use a fine grain film.	●Kodak Panatomic X ●Kodak Technical Pan 2415 ●Fuji Neopan F ●Agfapan 25 ●Ilford Pan F	Page 55
●A standard developer was used.	●Use a fine grain developer.	●Kodak Microdol D-23 D-25 ●Fuji Micro-Fine	
●Magnification ratio was too high.	●Use a large-format film.	●4″ × 5″ sheet film.	

Objects other than the specimen image appeared on the film

1. Shadow-like image

Cause	Correction	Remarks	Refer to page
●Optical path selector of the photographic attachment or trinocular tube was interrupted at some stage. 	●Engage the optical path selector at its proper position.		
●The field iris diaphragm was stopped down too much. 	●Open the field iris diaphragm a little wider than the photographed area of the finder eyepiece of the focusing telescope.		Page 23
●Tiny bits of film, dirt, etc. stuck to the prism of the photographic equipment or to the large-format relay lens.  Dirt on the prism	●Check for, and remove, dirt from the prism of the photographic attachment while the shutter is open (Time setting). ●Remove the large-format relay lens and clean it.	●Periodic checks are recommended if a large number of photographs is taken.	Pages 29-31

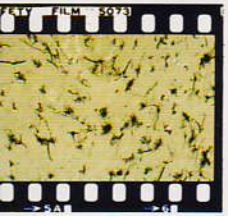
Cause	Correction	Remarks	Refer to page
●Dirt in the optical system  S Plan FL 1X	●Locate the dirt and remove it.	●You can locate the dirt by moving and rotating each checkpoint, alternately looking through the binocular tube, the focusing telescope of the photographic attachment and the film plane (by placing a piece of frosted glass in the camera body.)	Page 28 Pages 30~31

2. Sparks

Cause	Correction		Refer to page
●When moving the film or when unrolling the backing paper of the film, static electricity causes sparks.	●Do not rewind the film too rapidly. ●Keep humidity at 45% minimum in the room where you handle the film. ●Make sure that the camera back and the darkroom are free of dust.		

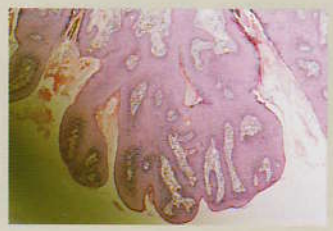
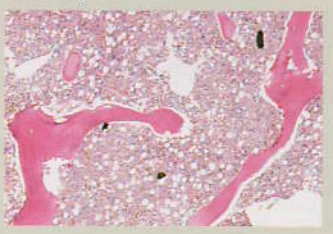
3. Reflection of window or room illumination

Cause	Correction		Refer to page
●Stray light enters from the eyepieces or the focusing telescope. 	●Move the optical path selector of the trinocular tube to the Camera 100% position and cover the focusing telescope of the photographic attachment with a cap. ●Put caps on both eyepieces and the focusing telescope of the photographic attachment. ●Set up the microscope in a different location.		Page 12



Uneven brightness

1. Uneven areas occur on one side of the frame, in the center, and under the perforation of the film

Cause	Correction	Remarks	Refer to page
●The microscope light source is not properly centered. 	●Properly adjust the light source.		
●The field iris diaphragm is off axis.	●Close the field iris diaphragm so that it appears in the visual field and adjust the condenser to center the diaphragm.		Page 21
●The optical system is contaminated by dirt. 	●Clean the optical system.		Pages 29 ~ 31
●Development problems (on black-and-white print)	●Develop the film properly.	●Be aware that a change in temperature occurs between the center and the periphery of the tank as a result of heat conduction by the steel tank.	Pages 64 ~ 65

2. Marks on the negative

Cause	Correction	Remarks	Refer to page
●Fixing time was too short or exhausted fixer was used.	●Increase fixing time or use a new fixer.		

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